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POPULATION DISTRIBUTION OF THE BEET LEAFHOPPER IN RELATION TO EXPERIMENTAL FIELD-PLOT LAY-OUT [1]

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INTRODUCTION

In field experiments to determine the relative merits of different insecticides or other treatments for controlling insect infestations, experimental designs such as the randomized block and the Latin square are frequently used. Such designs reduce the error of the experiment by restricting each comparison of unlike treatments to a limited part of the experimental field, thus giving treatment comparisons that are in a measure independent of location differences in the degree of infestation.

These designs are also well adapted to analysis of variance (*10*).[3] By this method of analysis the error reduction achieved by the design of the experiment may be evaluated and removed from the estimate of experimental error, thus increasing the precision with which the effects of treatment may be measured. Restricted designs are therefore justified on the general assumption that their use, together with the proper method of analysis, will significantly reduce the error variance, and thereby increase the efficiency of the experiment.

The nature of the distribution of the beet leafhopper (*Eutettix tenellus* (Baker)) in a particular field of sugar beets was studied on different dates, and with the data obtained from sampling beet-field populations, the relation between experimental design and the distribution of insect populations was determined.

MATERIALS AND METHODS

In May 1937 a field approximately 4½ acres in area near Grand Junction, Colo., planted with U. S. No. 34 sugar beets (*Beta vulgaris* L.) resistant to curly top was selected for the experiment. The field was divided into 36 equal-sized plots to form a 6×6 Latin square (fig. 1).

Each plot was 91 feet long and 60 feet (36 rows) wide, the rows being spaced 20 inches apart. The area of a plot was thus about ⅛

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[2] The author is indebted to W. C. Cook for suggestions and encouragement in the preparation of this manuscript, and to Myron E. Hall, who assisted in obtaining the field samples

[3] Italic numbers in parentheses refer to Literature Cited, p. 277.

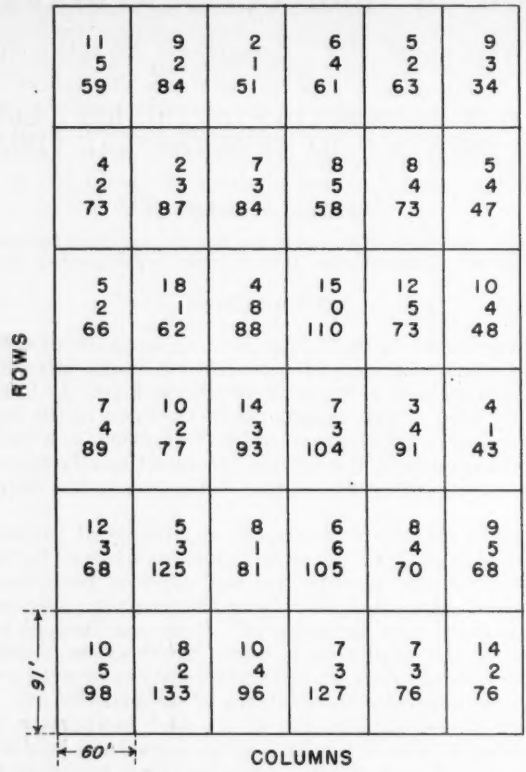

FIGURE 1.—Diagram of experimental field giving the total number of adult beet leafhoppers found on 10 beets in each plot on May 20 (upper figure); June 4 (middle figure); and August 26–27 (lower figure).

acre. At the time of thinning, the beets were spaced 10 inches apart along the rows.

Quantitative samples of the beet leafhopper population were obtained with a square-foot sampling cage essentially the same as that described by Hills (*14*). The first series of samples was taken in the field on May 20, 1937, shortly after the second influx of migrant beet leafhoppers into the Grand Valley fields, and about the time that the infestation was at its peak. The field was sampled again on June 4, when the migrant populations were on the decline, and again on August 26–27, after there had been brood development within the field. Ten square-foot cage samples were taken at random[4] in each plot on each date, or a total of 360 samples for the field. The adult leafhoppers were counted and recorded.

[4] Samples were chosen by moving about the plot with the head and eyes down, stopping at irregular intervals, and at each stop selecting the leafhoppers from the sixth beet along the row from the beet nearest the toe of the right foot.

The data obtained at each sampling were fitted to various theoretical distributions and tested by χ^2.

A study of the plot variation and the effect of local control in reducing experimental error was made by the method of analysis of variance. All of these analyses are on a single-sample basis.

NATURE OF THE DISTRIBUTION OF BEET LEAFHOPPER POPULATIONS

EARLY SEASON MIGRANT POPULATION

The nature of the sampling data suggests a discontinuous distribution such as is described by a Poisson series, a negative binominal (*11*, *21*, *22*), or Neyman's (*15*) contagious distribution, which has been tested for applicability to the field distribution of larval insects by Beall (*3*). Comparisons of the observed and the theoretical Poisson frequency distributions for the first two sampling dates are given in table 1. The χ^2 value in each case denotes a favorable agreement be-

TABLE 1.—*Distribution of 360 field samples classified according to the number of beef leafhoppers on a beet, 1937*

[Fitted Poisson distributions and test of goodness of fit]

Infestation class	May 20			June 4		
	Frequency		χ^2	Frequency		χ^2
	Observed	Calculated		Observed	Calculated	
0	174	161.30	0.9999	263	260.83	0.0180
1	112	129.48	2.3598	81	84.04	.1100
2	54	51.97	.0793	13	13.54	.0500
3	14	13.91		3	1.45	
4	4	2.79		0	.12	
5	1	.45	.4384	0	.02	
6	1	.06				
7+	0	.04				
Total	360	360.00	3.8774	360	360.00	.1780
Degrees of freedom			2			1
P_{χ^2}			.15			.68

tween the observed and the calculated values, and leads to the conclusion that at the time of the spring movement into the beet fields the distribution of the leafhopper is essentially in accordance with the Poisson law. This conclusion is supported by data given by Bowen [5] and by the analyses of an abundance of sampling data (unpublished) obtained in different years by different observers in the sugar beet areas in Idaho, western Colorado, Utah, and California.

The data in table 1 also agree closely with the negative binomial and the contagious distributions. Theoretical values for these distributions, however, are not included in table 1 for the reason that any sample distribution that fits the Poisson must also fit the negative binomial and the contagious. In practice the fit to the latter two usually will be closer than to the Poisson, because for these distribu-

[5] BOWEN, M. F. A METHOD OF ESTIMATING BEET LEAFHOPPER POPULATIONS FROM THE PROPORTION OF UNINFESTED PLANTS. U. S. Bureau Ent. and Plant Quar. Cir. ET–225, 6 pp., illus. 1945. [Processed.]

tions the observed data are forced to agree with theory in the total, the mean, and the variance, whereas for the Poisson, agreement is forced in only the total and the mean.

The variation among the plot totals (fig. 1) may be examined for general conformity to that of a Poisson distribution by the formula given by Fisher (*10*) and Rider (*17*),

$$\chi^2=\frac{\Sigma(x-\bar{x})^2}{\bar{x}}$$

χ^2 so calculated for the 36 plots on May 20 is 59.164, which for 35 degrees of freedom, corresponds to a probability near the 1-percent point.[6] This test therefore indicates a real tendency toward excessive variability in the data. A large contribution to χ^2 is supplied by the one plot in which a total of 18 leafhoppers was recorded. If this plot is omitted from the calculations, the variance is not excessive. χ^2 then equals 46.785, which for 34 degrees of freedom corresponds to a probability of 0.14, approximately. The analysis of variance of the $\frac{1}{8}$-acre plots (table 4), which denotes a difference between plots barely exceeding the 5-percent level of significance, also suggests a greater variability among the plot means than would be expected if the distribution of the leafhopper were purely random. Bliss (*4*), in an analysis of data obtained by Fleming and Baker (*12*) on the distribution of Japanese beetle larvae, shows a highly significant variation between plots over an apparently uniform section as small as 18×20 feet (0.008 acre). This condition, observed by Bliss, is more conclusive than appears for the migrant beet leafhopper population here considered, notwithstanding that the experimental area studied was very much larger (4.5 acres) in the case of the leafhopper.

The preceding discussion indicates that there is a tendency for the migrant population of May 20 to depart from a Poisson distribution. This tendency is toward an excess of high and low counts similar to that observed much more distinctly in the resident population of August 26–27, which will be discussed later.

It is reasonable to expect that the true distribution of migrant populations of the beet leafhopper will depart from a Poisson series, because of the many factors both physical and biological that may operate to destroy a perfectly independent distribution of the insect. Air currents or a preference by the leafhopper for certain plots within the field might have caused an uneven distribution of the population on May 20. If this were so, it seems logical to assume that the plot differences would persist during the short period from May 20 to June 4, and that a positive correlation would exist between the plot counts made on these two dates. A cursory examination of the plot totals in figure 1 indicates that no such relationship exists. Actually the correlation coefficient is negative; for the 36 pairs of counts $r=-0.16$, a nonsignificant value, indicating the absence of any relationship between the plot totals of the first two sampling dates. This absence of a significant correlation suggests that the comparatively large number of leafhoppers taken in some of the plots on May 20 might have resulted from chance variations of sampling rather than from a real preference by the insect for certain parts of the field, or from

[6] For degrees of freedom exceeding 30 the expression $\sqrt{2\chi^2}-\sqrt{2n-1}$ is assumed to be a normal deviate with unit standard error.

the effects of physical factors, such as air currents, that may have deposited more leafhoppers in some locations than in others.

The sample distribution of June 4 shows a closer agreement with the Poisson law than does that of May 20, but from the data available it cannot be determined whether this agreement is in fact better. The principal evidence derived from a study of both distributions indicates that the agreement with the Poisson law is essentially satisfactory.

The natural mortality of migrant beet leafhoppers is high. For this reason a decrease in migrant populations always occurs after the spring movement into the fields has ceased, or when this movement is not sufficient to offset the natural mortality in the population already present. Thus, in the field here considered, the leafhopper population decreased from a mean of 0.803 per beet on May 20 to 0.322 per beet on June 4. The fact that on both dates the distribution was essentially in accordance with the Poisson law denotes that the factors affecting mortality of the beet leafhopper acted uniformly in the different plots.

LATER SEASON RESIDENT POPULATION

An attempt to fit the data of August 26–27 to a Poisson series was not successful. This is illustrated by comparing the observed with the calculated Poisson law values given in table 2, and by the corresponding graph in figure 2. In the same table and figure the later season data are also fitted to the negative binomial and the contagious distributions.

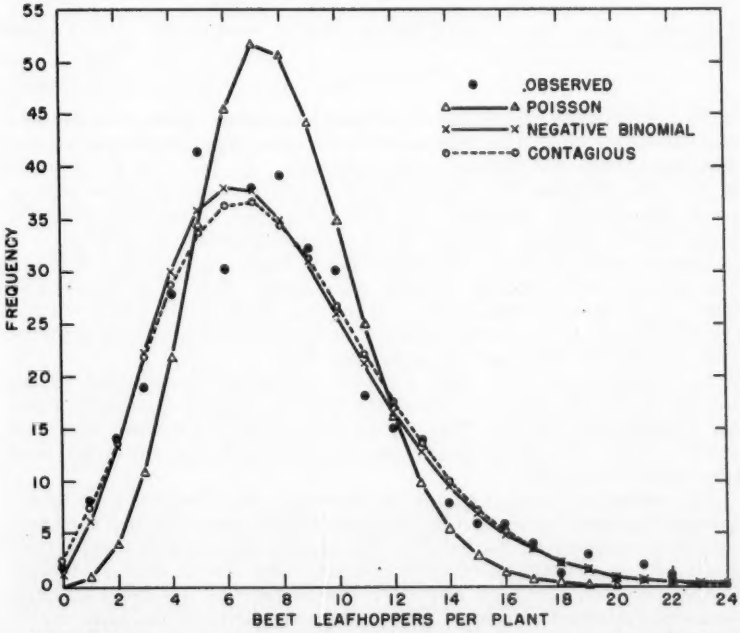

FIGURE 2.—Observed and theoretical Poisson, contagious, and negative binomial frequency distributions of the number of beet leafhoppers per beet in the field samples of August 26–27, 1937. Data from table 2.

TABLE 2.—*Distribution of 360 field samples classified according to the number of beet leafhoppers on a beet, August 26–27, 1937*

[Fitted Poisson, contagious, and negative binomial distributions, and test of goodness of fit]

Infestation class	Frequency				χ^2		
	Observed	Calculated					
		Poisson	Contagious	Binomial	Poisson	Contagious	Binomial
0	2	0.13	2.68	1.62	64.4432	0.0019	0.5765
1	8	1.06	7.46	6.25			
2	14	4.19	14.26	13.66		.0047	.0085
3	19	11.02	21.78	22.24	5.7786	.3548	.4720
4	28	21.75	28.72	29.98	1.7960	.0180	.1308
5	41	34.33	33.78	35.41	1.2959	1.5432	.8825
6	30	45.15	36.46	37.86	5.0836	1.1446	1.6318
7	38	50.90	36.63	37.46	3.2694	.0512	.0078
8	39	50.21	34.65	34.82	2.5028	.5461	.5018
9	32	44.03	31.13	30.75	3.2869	.0243	.0508
10	30	34.75	26.72	26.00	.6493	.4026	.6154
11	18	24.93	22.04	21.20	1.9264	.7405	.4830
12	15	16.39	17.53	16.75	.1179	.3651	.1828
13	14	9.95	13.50	12.87	1.6485	.0185	.0992
14	8	5.61	10.08	9.65	1.0182	.4292	.2821
15	6	2.95	7.33	7.08		.2413	.1647
16	6	1.46	5.20	5.10		.1231	.1588
17	4	.68	3.60	3.61			
18	1	.30	2.44	2.52			
19	3	.12	1.62	1.73			
20	1	.05	1.06	1.17	60.4571	.3784	.0434
21	2	.02	.68	.79			
22	1	.01	.43	.52			
23	0			.34			
24+	0	.01	.22	.62			
Total	360	360.00	360.00	360.00	153.2738	6.3875	6.2919
Degrees of freedom					12	14	14
P_{χ^2}					<.001	.954	.957

The data show a highly significant departure from the Poisson distribution, while the agreement with the negative binomial and the contagious distribution is satisfactory. In fact the χ^2 criterion in the case of these two distributions indicates an even closer agreement between the observed and the calculated values than usually appears for random samples from a homogeneous population. χ^2 values as small as those obtained for the negative binomial and the contagious would appear usually only about once in 23 trials and once in 22 trials respectively. However, such small values might credibly result from chance, and the fit to these distributions is therefore regarded as being not unreasonably close.

It is worthy of note that for this particular sample the values calculated for the negative binomial and the contagious distributions are remarkably similar. Some of Beall's (*3*) data likewise have been found to show satisfactory agreements with both the contagious and the negative binomial.

The contagious and the negative binomial distributions may be regarded as generalizations of the Poisson law for cases in which the variability is excessive as compared to the expectations of a Poisson series. Both of these distributions are characterized by an excess of high and low counts, and both approach the Poisson as the mean and the variance approach equality. The contagious distribution, however, is more flexible than the negative binomial and may sometimes be bimodal, whereas the negative binomial is never bimodal. The negative

binomial is much easier to calculate than the contagious especially when the number of infestation classes is large.

In fitting the observed distributions to a negative binomial $(q-p)^{-n}$, the values of q, p, and n were derived by calculation from the empirical data following the method given by Fry (*13*), i. e., $p=1-V/\bar{x}$, $q=1-p$, and $n=\bar{x}/p$; where V is the variance of the distribution and $\bar{x}$ is the mean infestation per plant. Obviously, the binomial will be negative or positive respectively as the variance is greater or less than the mean. In some samples of migrant beet leafhopper populations, positive binomials ($V<\bar{x}$) have appeared, but in such samples p did not differ significantly from zero (*22*) and the data, therefore, were considered to be in essential agreement with the Poisson law.

In practice, departures from the Poisson will usually be such as to make V greater than $\bar{x}$, although under certain conditions, such as very dense insect populations where there is overcrowding and competition for the available space, a distribution more uniform than the Poisson might result. "Student" (*21*) has studied the effect of departures from the conditions that lead to Poisson's law, and has indicated the conditions in which data may be expected to conform more closely to a negative binomial than to a Poisson series.

The contagious distribution[7] was fitted by using the recurrent formula given by Neyman (*15*) and Beall (*3*),

$$P_{(x=n+1)}=\frac{m_1 m_2 e^{-m_2}}{n+1}\sum_{t=0}^{n}\frac{m_2{}^t}{t!}\,P_{(x=n-t)}$$

where the initial value, $P_{(x=0)}=e^{-m_1(1-e^{-m_2})}$

The parameters m_1 and m_2 of the above formulae are estimated from the mean and the variance as $\bar{x}^2/(V-\bar{x})$ *and* $(V-\bar{x})/\bar{x}$, respectively.

Later season populations of the beet leafhopper do not agree with Poisson expectations, probably because field conditions at this time do not favor an independent random distribution. Individual plants or certain locations within the field may be more attractive to, or favor a more rapid development of, the insect than others, which condition would upset an independent random distribution and cause the excesses in the small and the large frequency classes (see table 2 and fig. 2) that usually have been observed in distributions that departed from a Poisson series. Part of the contagiousness observed in the resident beet leafhopper population may be attributed to a random dispersion of adults which developed from groups of eggs deposited by the spring migrants. It is doubtful, however, whether this is the primary cause of contagiousness in the field distribution of an insect as active as the beet leafhopper.

The variation in the leafhopper population on August 26–27 cannot be explained by differences in the initial infestation, which, as the analyses of the migrant populations have shown, was essentially uniform. Correlation analyses support this view. The correlation coefficient between the plot totals of May 20 and those of August 26–27

[7] Contagious distribution of type A depending on two parameters. See Neyman (*15*, pp. 45–48).

was +0.0258, and between the plot totals of June 4 and those of August 26–27 it was +0.0260. Both correlation coefficients are nonsignificant, an indication of the absence of relationship between the initial infestations and the populations that subsequently developed in the different plots within the field.

Data obtained from a field of sugar beets at the Asarco farm, Magna, Utah, in the summer of 1932 illustrate this point. Samples taken in this field on June 20 and 28 showed satisfactory agreement with the Poisson law, and denoted that early in the season the distribution of the leafhopper was quite uniform. Later, however, distinct populations developed in sections of the field characterized by beets of different growth types. This was shown by sampling data taken at weekly intervals from August 6 to 25, inclusive. These data, summarized in table 3, consistently show a significant inverse relationship between the

TABLE 3.—*Beet leafhopper populations on beets of different growth type in field at the Asarco farm, Magna, Utah, 1932*

Date	Leafhopper population per beet [1]			Difference between means		
	Large beets (M_1)	Medium large beets (M_2)	Small wilted beets (M_3)	M_3-M_1	M_3-M_2	M_2-M_1
Aug. 6	1.44±0.21	3.60±0.44	8.48±0.88	7.04±0.90	4.88±0.98	2.16±0.49
11	1.92± .26	4.20± .44	15.44±1.53	13.52±1.55	11.24±1.59	2.28± .51
18	2.52± .33	6.52± .70	23.32±2.13	20.80±2.16	16.80±2.24	4.00± .77
25	3.28± .33	[2]	31.32±2.75	28.04±2.77		

[1] Each mean based on counts from 25 beets.
[2] Rain prevented sampling.

density of the leafhopper population and the size of the beets. It is not definitely known just what environmental factors operated to produce the disparate populations on the different-sized beets, but it seems logical to believe that the higher temperatures associated with the small wilted beets favored the production of large leafhopper populations.

In this connection it might be noted that soil heterogeneity may greatly influence the distribution of insect populations indirectly through its action on the plants upon which the insect lives.

VARIATION BETWEEN PLOTS AND WITHIN PLOTS

EARLY SEASON MIGRANT POPULATION

The population data for the 36 plots may be combined to form plots of various sizes and shapes. Twelve size-shape combinations were studied; these are illustrated in figure 3. The variances within and between plots for the data collected on May 20 were computed for each arrangement and are given in table 4.

These analyses were made to determine the effect of plot size on the within-plot variation, which is a measure of the sampling error, and to test whether population heterogeneity was a significant factor affecting leafhopper numbers in the different plots. There is a tendency for the within-plot variance to increase as the subplots become larger, but the effect is small, and the chief evidence is that altering the size or shape of the plot did not materially influence the sampling error.

TABLE 4.—*Analysis of variance of different-sized subplots for field samples of May 20, 1937*

Shape and size of plots		Variance [1]		*F*
Length×width	Area	Between plots	Within plots	
Feet	*Acres*			
91×60	1/8	1.357 (35)	0.924 (324)	1.47
182×60	1/4	1.191 (17)	.955 (342)	1.25
91×120	1/4	1.267 (17)	.952 (342)	1.33
273×60	3/8	1.027 (11)	.965 (348)	1.06
91×180	3/8	1.548 (11)	.948 (348)	1.63
(2)	3/8	1.445 (11)	.951 (348)	1.52
182×120	1/2	1.015 (8)	.965 (351)	1.05
273×120	3/4	.263 (5)	.976 (354)	3.71
182×180	3/4	1.203 (5)	.963 (354)	1.25
273×180	1 1/8	1.218 (3)	.964 (356)	1.26
546×180	2 1/4	.025 (1)	.969 (358)	38.76
273×360	2 1/4	.225 (1)	.969 (358)	4.31

[1] Numbers in parentheses denote degrees of freedom.

[2] 182×60+91×60.

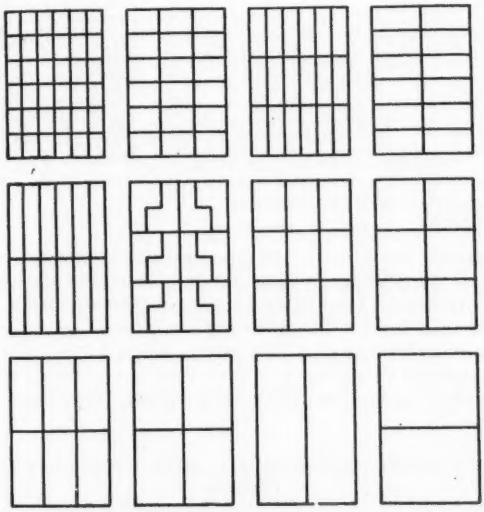

FIGURE 3.—Some of the various sizes and shapes of plots obtained by different combinations of the original 36 plots.

The significance of the difference between plots was determined by the *F* test (*19*). The between-plot variance is barely significant for the 1/8-acre plots, but in no other instance does the observed value of *F* exceed that required at the 5-percent level of significance. The variation between plots was not significantly greater than that within plots, an indication that for the migrant population of May 20 population heterogeneity was not a significant factor affecting the infestation in the different plots. This shows a remarkable degree of uniformity in the leafhopper infestation at this particular time, and corroborates the findings of table 1 which indicate a Poisson distribution.

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The data of June 4 show essentially the same condition as those of May 20, and therefore are not considered in detail.

LATER SEASON RESIDENT POPULATION

The population of August 26–27, which resulted largely from the reproductive activity of leafhoppers that migrated into the field prior to June 1, contrasts with the population of May 20. The analyses of variance between plots and within plots for the different arrangements on August 26–27 appear in table 5. The observed value of F for each

TABLE 5.—*Analysis of variance of different-sized subplots for field samples of August 26–27, 1937*

Shape and size of plots		Variance[1]		F
Length×width (feet)	Area	Between plots	Within plots	
	Acres			
91×60	1/8	55.17(35)	11.96 (324)	4.61
182×60	1/4	100.32(17)	11.99 (342)	8.37
91×120	1/4	75.86(17)	13.20 (342)	5.75
273×60	3/8	113.82(11)	13.08 (348)	8.70
91×180	3/8	72.27(11)	14.40 (348)	5.02
[2]	3/8	108.93(11)	13.24 (348)	8.23
182×120	1/2	148.12 (8)	13.32 (351)	11.12
273×120	3/4	180.69 (5)	13.84 (354)	13.06
182×180	3/4	129.32 (5)	14.57 (354)	8.88
273×180	1 1/8	179.94 (3)	14.79 (356)	12.17
546×180	2 1/4	97.14 (1)	15.94 (358)	6.09
273×360	2 1/4	442.22 (1)	14.98 (358)	29.52

[1] Numbers in parentheses denote degrees of freedom.
[2] Irregular, 182×60+91×60.

comparison except one (546 × 180) exceeds the value required at the 1-percent level of significance, which indicates a highly significant difference between plots, or a marked degree of heterogeneity in the field infestation at this time. There was an appreciable reduction in the sampling error with decreased plot size. Apparently the distribution of the leafhopper at this time was such that for plots of the same size the sampling error is smaller in the long narrow plots than in the short wide ones.

EFFICIENCY OF LOCAL CONTROL IN REDUCING EXPERIMENTAL ERROR

In field-plot experiments involving different treatments the estimate of the error in the experiment is derived from the variation between plots. To illustrate, the total sum of squares between plots in a Latin-square arrangement may be apportioned to the known and controlled causes of variation, i. e., rows, columns, and treatments, the residual sum of squares being due to unknown causes, or experimental error. The variance due to any cause is then determined by dividing the sum of squares by the corresponding number of degrees of freedom.

Table 6 gives a summary of the between-plot analyses of variance of the different-sized plots for the sampling data of the migrant leafhopper population of May 20. Table 7 gives a similar summary for the sampling data of the later season population of August 26–27.

TABLE 6.—*Summary of between-plot analyses of variance for plots of different sizes and shapes and the efficiency of local control in reducing experimental error for data of May 20, 1937*

Treatments × replications	Shape and size of plots		Variance [1]						Error change due to local control.
	Length × width	Area	Total	Rows	Columns	Treatment [2]	Error	Treatment + error	
	Feet	*Acres*							*Percent*
6×6	91×60	1/6	1.357 (35)	1.869 (5)	0.203 (5)	1.223 (5)	1.551 (20)	1.485 (25)	+9.43
6×3	182×60	1/4	1.191 (17)	2.636 (2)		1.123 (5)	.936 (10)	.998 (15)	−16.20
3×6	182×60	1/4	1.191 (17)		.203 (5)	.144 (2)	1.894 (10)	1.603 (12)	+34.59
6×3	91×120	1/4	1.267 (17)		.136 (2)	.729 (5)	1.763 (10)	1.418 (15)	+11.92
3×6	91×120	1/4	1.267 (17)	1.869 (5)		.136 (2)	1.193 (10)	1.017 (12)	−19.73
6×2	273×60	3/8	1.027 (11)	.225 (1)		1.656 (5)	.558 (5)	1.107 (10)	+7.79
2×6	273×60	3/8	1.027 (11)		.203 (5)	.803 (1)	1.896 (5)	1.714 (6)	+66.89
6×2	91×180	3/8	1.548 (11)		.025 (1)	.643 (5)	2.758 (5)	1.700 (10)	+9.82
2×6	91×180	3/8	1.548 (11)	1.869 (5)		1.469 (1)	1.243 (5)	1.280 (6)	−17.31
4×3	[3]	3/8	1.445 (11)	2.636 (2)		.506 (3)	1.518 (6)	1.180 (9)	−18.34
3×4	[3]	3/8	1.445 (11)		.632 (3)	.386 (2)	2.205 (6)	1.750 (8)	+21.11
3×3	182×120	1/2	1.015 (8)	2.636 (2)	.136 (2)	.620 (2)	.670 (2)	.644 (4)	−36.55
3×2	182×180	3/4	1.203 (5)		.025 (1)	.720 (2)	2.275 (2)	1.497 (4)	+24.44
2×3	182×180	3/4	1.203 (5)	2.636 (2)		.625 (1)	.058 (2)	.247 (3)	−79.47
3×2	273×120	3/4	.263 (5)	.225 (1)		.544 (2)	<.001 (2)	.272 (4)	+3.42
2×3	273×120	3/4	.263 (5)		.136 (2)	.336 (1)	.353 (2)	.347 (3)	+31.94
2×2	273×180	1 1/8	1.218 (3)	.225 (1)		3.403 (1)	.025 (1)	1.714 (2)	+40.72
2×2	273×180	1 1/8	1.218 (3)		.025 (1)	.225 (1)	3.403 (1)	1.814 (2)	+48.93

[1] Numbers in parentheses denote degrees of freedom.
[2] Hypothetical.
[3] Irregular, 182×60+91×60.

TABLE 7.—*Summary of between-plot analysis of variance for plots of different sizes and shapes and the efficiency of local control in reducing experimental error for data of August 26–27, 1937*

Treatments×replications	Shape and size of plots		Variance [1]						Error change due to local control
	Length×width	Area	Total	Rows	Columns	Treatment [2]	Error	Treatment + error	
	Feet	*Acres*							*Percent*
6×6	91×60	1/8	55.17 (35)	127.06 (5)	145.55 (5)	18.26 (5)	23.83 (20)	22.71 (25)	−58.84
6×3	182×60	1/4	100.32 (17)	253.81 (2)	----------	96.82 (5)	71.37 (10)	79.85 (15)	−20.40
3×6	182×60	1/4	100.32 (17)	----------	145.55 (5)	118.30 (2)	74.11 (10)	81.47 (12)	−18.79
6×3	91×120	1/4	75.86 (17)	----------	216.76 (2)	31.56 (5)	69.84 (10)	57.08 (15)	−24.76
3×6	91×120	1/4	75.86 (17)	127.06 (5)	----------	34.36 (2)	58.57 (10)	54.54 (12)	−28.10
6×2	273×60	3/8	113.82 (11)	442.22 (1)	----------	59.88 (5)	102.09 (5)	80.99 (10)	−28.84
2×6	273×60	3/8	113.82 (11)	----------	145.55 (5)	1.74 (1)	104.52 (5)	87.39 (6)	−23.22
6×2	91×180	3/8	72.27 (11)	----------	97.14 (1)	28.63 (5)	110.94 (5)	69.79 (10)	−3.43
2×6	91×180	3/8	72.27 (11)	127.06 (5)	----------	40.67 (1)	23.81 (5)	26.62 (6)	−63.16
4×3	(3)	3/8	108.93 (11)	253.81 (2)	----------	93.83 (3)	68.20 (6)	76.74 (9)	−29.55
3×4	(3)	3/8	108.93 (11)	----------	188.76 (3)	108.22 (2)	69.26 (6)	79.00 (8)	−27.48
3×3	182×120	1/2	148.12 (8)	253.81 (2)	216.76 (2)	10.86 (2)	95.03 (2)	52.94 (4)	−64.26
3×2	182×180	3/4	129.32 (5)	----------	97.14 (1)	140.81 (2)	133.93 (2)	137.37 (4)	+6.22
2×3	182×180	3/4	129.32 (5)	253.81 (2)	----------	.80 (1)	69.10 (2)	46.33 (3)	−64.17
3×2	273×120	3/4	180.69 (5)	442.22 (1)	----------	72.91 (2)	157.71 (2)	115.31 (4)	−36.18
2×3	273×120	3/4	180.69 (5)	----------	216.76 (2)	23.00 (1)	223.47 (2)	156.65 (3)	−13.30
2×2	273×180	1 1/8	179.94 (3)	442.22 (1)	----------	.47 (1)	97.14 (1)	48.80 (2)	−72.88
2×2	273×180	1 1/8	179.94 (3)	----------	97.14 (1)	.47 (1)	442.22 (1)	221.34 (2)	+23.01

[1] Numbers in parentheses denote degrees of freedom.
[2] Hypothetical.
[3] Irregular, 182×60+91×60.

Inasmuch as the effect of local control is the primary concern of this study, the variances due to rows and columns are of chief importance. No treatments were applied, and the experiment may be regarded as a uniformity trial. Therefore, the treatment effects are purely hpothetical and are presented in these tables only to show the adequacy of the experimental design. It will be noted that seldom is there indicated a significant treatment response. Occasional significant deviations can be expected in a large series of samples even when drawn from homogeneous material. Where only a few large plots are concerned, the treatment effects might be confounded with location differences arising from an unfortunate chance assignment of the treatments to the various plots. Such must be the explanation of the statistically significant treatment effect indicated in line 15 of table 6, and again in the last line of table 7, where there appears to be a significant negative intraclass correlation. Since the treatments were hypothetical the appropriate estimate of experimental error, by which to judge the effects of local control, is obtained by adding the sums of squares for treatment and error and dividing by the combined degrees of freedom. This figure is given in the column headed "Treatment + error."

The effect of local control is measured by the percentage change in the error variance due to the removal of the contributions of rows and/or columns (blocks) from the total variance.

EARLY SEASON MIGRANT POPULATION

Early in the season a field of sugar beets offers an apparently uniform environment. The beets are small and the effect of soil heterogeneity has not yet greatly differentiated them. Previous analyses, given in tables 1 and 4, denoted that on May 20 the leafhopper infestation over the experimental field was essentially uniform, and, therefore, the usefulness of local control when applied to it was highly questionable.

Table 6 shows that restricted designs resulted in an increase in the estimate of experimental error in 12 of the 18 arrangements. In those arrangements where a decrease is indicated, the utility of local control is doubtful, because the variation between blocks usually is not significant and may have resulted from chance. The negative value of restriction, when applied to the migrant beet leafhopper population of May 20, is further emphasized by the fact that the nonsignificant change in the error variance is accompanied by a reduction in the degree of freedom available for the estimate of experimental error. In this connection it should be noted that there was a 66.89 percent increase in the error variance due to the removal of the columns component in the 2×6 randomized blocks design (table 6). The variance of 1.027 is increased by this arrangement to 1.714, and in the process 5 degrees of freedom corresponding to blocks were sacrificed. Clearly the design was very unsuitable.

Although the between-plot analyses for the various arrangements indicate little or no significant effect due to local differences in the leafhopper infestation, it is interesting to compare the row and column variances for plots of the same size and shape. In every arrangement the variation between columns is smaller than that between rows, a

fact suggesting a more uniform distribution of the leafhopper in one direction of the field than in the other. Such a distribution would be likely to develop from an influx of a weak flying species moving from a source along one side of a field, the resulting infestation being heaviest on the side adjacent to the source and gradually diminishing toward the far side.

The beet leafhopper is not a weak flier. Agricultural areas may be infested by dispersals of this insect from breeding sources sometimes hundreds of miles distant (*7*, *18*). It also attains considerable altitude during its flights. Annand and associates (*1*) have shown that during dispersals in southern Idaho large numbers may be found as high as 25 feet at least, and it is not unlikely that much greater altitudes are common. Both of these flight characteristics of the beet leafhopper tend to favor a uniform infestation at the time of dispersal. Over large fields, or very long fields, location differences in the degree of infestation may be considerable. Certainly different fields in the same general area will exhibit highly significant differences in population density. Among other factors, wind currents undoubtedly play an important role in this local distribution, but for areas as small as most sugar-beet fields the effect is apparently small.

From a comparison of the effect of the removal of the row and column variances in the different arrangements it is evident that when a decrease in the error variance occurs it originates from the elimination of the contribution of rows; when an increase occurs it is smaller for the rows than it is for the columns. From this it appears that if a restricted random arrangement were to be used at all in this field at this particular time, the best results would be obtained from randomized blocks laid out across the field in the row direction, not in the column direction.

However, the principal evidence from the analyses of the data of May 20 is that restricted-random arrangements are of little value in field tests made on migrant beet leafhopper populations. This statement probably will apply generally because of the uniformity of the distribution of the leafhopper during the time of the spring flights into the beet fields.

Observations in many fields other than the one considered in this paper have indicated that migrant beet leafhoppers are distributed in a Poisson series. This distribution implies an equal probability of infestation in each of the plots. If the infestations in the plots are alike, it follows that the infestations in different combinations of them will also be alike, and restricted arrangements such as the Latin square or randomized blocks cannot, under such conditions of uniformity, accomplish the purpose for which they are intended.

Migrant leafhopper densities, ranging from a mean of 0.01 to 5 per beet, have been found in essential agreement with the Poisson distribution. At higher densities this distribution law begins to break down owing to an excess of high and low counts, probably because some plants are more attractive to the insect than others. A differential attractiveness among the plants, however, will not necessarily destroy the similarity of the plot infestations, provided the more attractive plants are randomly distributed over the field. This seems to apply to migrant leafhoper populations, so that even at high population densities the practical utility of local control is doubtful.

Further studies, however, are necessary to confirm or refute this belief.

Where migrant populations of the beet leafhopper are concerned it appears that the most satisfactory results will be obtained by a complete randomization of the treatment replications.

LATER SEASON RESIDENT POPULATION

The later season resident population was composed largely of progeny of the spring migrants that moved into the field before June 1. By late August the beets in a field usually manifest obvious differences in growth, and the leafhopper environment is much less uniform than it is early in the season. The environmental differences that develop within the field apparently are reflected in a marked heterogeneity in the leafhopper population.

Population differences associated with types of beet growth have commonly been observed by workers on the beet leafhopper project. Such differences are clearly illustrated by the data obtained at Magna, Utah, in 1932 (table 3). When such obvious differences appear in an experimental field, the investigator might readily take advantage of the associated differences in the leafhopper infestation by orienting the experiment to conform to the various types of plant growth.

Visual orientation of the plots, however, is not always practicable, since marked differences in the leafhopper population may and do appear in the absence of any clearly defined types of plant growth. Such was the case on August 26–27 in the field here considered. Although definite heterogeneity existed in the leafhopper infestation at this time (tables 5 and 7) it would have been impossible, by visual inspection of the field, to associate this heterogeneity with variations in the type of beet growth. Usually it is only by methods of sampling that infestation differences can be recognized with reasonable accuracy.

From table 7 it is evident that local control effected a substantial reduction in the estimate of experimental error, when applied to the resident leafhopper population of August 26–27. In practically all arrangements a decrease in the error variance resulted from removing the variation between rows or columns. In the 6 × 6 Latin square, for example, eliminating the infestation differences between rows and columns reduced the error variance by 58.8 percent; thus, this arrangement would have more than doubled the sensitivity of an experiment as a means of detecting treatment differences.

It is interesting to compare the percentage reduction in the error variances effected by removing rows or columns, when plots of the same shape and size are considered. Removal of the row contribution produced the larger decrease for every arrangement except the 6 × 6 Latin square. In a general way this parallels conditions observed in the data of May 20 except that then the effect of rows, although usually not significant, was larger than that of columns in every arrangement, the 6 × 6 Latin square included.

Although for the data of August 26–27 the rows component was somewhat smaller than that of columns in the 6 × 6 Latin square, in every other arrangement the rows effect was more pronounced. This

is explained by the presence of a definite population gradient across the field in the direction affected by the rows, so that combining the plots in this direction never decreased the differences between rows. There was no definite gradient in the direction affected by the columns, and in combining the plots in this direction the effect sometimes has been to decrease the relative differences. Thus, for the plot 180 feet wide, the error variance was actually increased by eliminating the variation between columns in two instances (182×180 and 273×180), and only an inappreciable reduction occurred in the other (91×180).

DISCUSSION

This study has demonstrated the relationship of insect-population distribution to experimental design. In experiments involving migrant beet leafhopper populations, restricted-random arrangements designed to reduce the experimental error are of little or no value. This results from the uniform distribution of the leafhopper over the experimental area at the time of the spring dispersal into the beet fields. Later season populations exhibit considerable heterogeneity, and the value of local control to increase the accuracy of the results is unmistakable.

As a general rule, in field experiments with any insect species the advantage gained from restricted random arrangements will vary directly with the degree of heterogeneity exhibited by the population sampled. The object of restriction is to include in the location differences as much of the total variation as possible. Obviously this may be accomplished most readily when the population heterogeneity is expressed as a gradient in one or two directions across the experimental field, or lies in definite zones, rather than in patches scattered over the field.

Preliminary samples are usually taken in entomological field tests. Analyses of the preliminary data should indicate the plot arrangement that will most effectively increase the precision of the experiment.

Theoretically, the method of analysis of variance is based on the assumption of a normal distribution. Often this assumption does not hold for field sampling data. Since the distribution of migrant beet leafhoppers is described by the Poisson law, the application of analysis of variance to it is open to question. A similar objection might be made to applying analysis of variance to the later season resident population of August 26–27, which deviates significantly from the normal but conforms favorably with the negative binomial and the contagious distributions. For all practical purposes, however, the variance method applied to tests of significance will probably give reliable results despite a considerable degree of skewness in the data. Experimental studies on nonnormal data by Pearson (*16*) and by Eden and Yates (*9*) support this view. More specifically, Chapman (*5*) has demonstrated a satisfactory agreement between the observed and the theoretical distribution of Z for samples drawn from a Poisson distribution having a mean of 1.0. This approximates the mean (0.803) of the sample distribution of May 20 considered in this report.

In addition to the assumption of a normal distribution, the validity of the generalized standard error derived from a combined analysis of variance is based on the assumption that although the different elements of the population may have different means their variance must be the same. Clearly this assumption may be violated for data of the Poisson type. The variance in a Poisson distribution is not independent, but is equal to the mean. Thus if population heterogeneity is involved, or if different treatments exert a significant effect, the corresponding means, and therefore the variances, will be different, and a combined analysis of the data will be unwarranted.

Considerable caution should be exercised in the analysis and interpretation of such data; otherwise, misleading conclusions may be drawn. Bartlett (*2*) and Cochran (*6*) have dealt at length with this aspect of the problem, and for data of the Poisson type they suggest a square-root transformation to equalize the variances before proceeding with the analysis. For plot counts between 10 and 100 they suggest the simple square-root ($\sqrt{x}$) transformation, and for plot totals the majority of which are under 10 the transformation $\sqrt{x+1/2}$ is recommended.

In the present study, which deals with uniformity data, differential variability was negligible in the populations of May 20 and June 4. A transformation of the data was therefore considered unnecessary. If different treatments had been employed in the experimental field on these dates, then differential variability probably would have been introduced by the treatments, and the $\sqrt{x+1/2}$ transformation would have been appropriate since the plot totals (fig. 1) were below 10 in most cases.

Analyses of variance have been made on the original data and on the transformed data with essentially the same results. Take, for example, the data for the 6×6 Latin square on May 20. When the original figures are used the F values for rows, columns, and treatments respectively are 1.26, 7.32, and 1.27; when the $\sqrt{x+1/2}$ transformation is used the corresponding F values are 1.17, 7.93, and 1.51. Similarly, for the data of the 6×6 Latin square on June 4, when the original data are used the F values for rows, columns, and treatments respectively are 4.49, 1.79, and 1.42; and when the transformation is used the corresponding F values are 4.43, 1.97, and 1.42. Practically the same conclusion regarding levels of significance would be drawn from the analyses of the original data as from the analyses of the transformed data.

The data obtained on August 26–27, besides being non-normal, showed definite heterogeneity and, like those of the first two samplings, are of a type such that the variance and the mean are related. The differences in variability, however, are not so great as to affect appreciably the inferences drawn from the results of analyses performed on the original scale. The analysis of the data for the 6×6 Latin square, when repeated with the simple square-root ($\sqrt{x}$) transformation, gives F values of 5.94, 7.15, and 1.35 for rows, columns, and treatments respectively. These compare with corresponding F values of 5.59, 6.41, and 1.31 when the original figures are used. Both analyses give essentially the same result concerning the significance of the difference between rows, columns, and treatments.

The data considered in this paper further illustrate the practical applicability of the method of analysis of variance to skew (non-normal) distributions (*5*, *9*, *16*), although, by this, it is not intended to imply that a suitable transformation may not often be useful, or even quite necessary, to evaluate correctly the results of an experiment analysed by the variance method.

In this paper field-plot lay-out has been viewed solely from the standpoint of insect-population distribution. Obviously other considerations may be of importance. For example, if it is intended that different insecticides shall be studied, not only as they directly affect the insect but also according to the indirect effect of different degrees of kill on the resulting crop yield, then soil heterogeneity also must be considered. Under this condition restricted-random arrangements would seem advisable, despite uniformity of the insect infestation.

Insect activity and movement may render impracticable the use of designs that otherwise would be very helpful. This difficulty is illustrated by the work of Douglass, Wakeland, and Gillette (*8*) on field experiments for the control of the beet leafhopper in southern Idaho. They showed that a satisfactory kill could be obtained with a pyrethrum oil spray. However, neither the incidence of curly top disease, which is transmitted by the leafhopper, nor the yield of beets per acre in the sprayed plots was significantly different from that in the unsprayed check plots. The beet leafhopper is very active and interplot movements and dispersals into the field after the insecticides were applied completely obscured the effects of the treatment when these were measured by reductions in curly top disease or by increases in the yield of beets. Steiner (*20*) encountered a similar difficulty when he used small plots for field experiments with insecticides for the control of the codling moth. He found that because of intertree and interplot movements of the adult moths, treatment effects might be largely obscured if based only on records from drop and harvest fruit.

SUMMARY

The relation of insect-population distribution to experimental design has been studied by analyses of uniformity data obtained from sampling field populations of the beet leafhopper. (*Eutettix tenellus* (Baker)).

The distribution of the beet leafhopper at the time of the spring dispersal into the sugar-beet fields is in essential agreement with the Poisson law. Later season resident populations apparently conform to the negative binomial and the contagious distributions.

The variation between plots was not significantly greater than the variation within plots for the migrant leafhopper populations, which indicates that population heterogeneity was not a significant factor affecting the infestation in the different plots. The later season resident population showed highly significant differences between the plot infestations.

Restricted random designs gave little or no reduction in error variance when applied to the migrant beet leafhopper populations, because of the uniformity of the distribution of the insect over the

experimental field. These designs, however, effected a significant reduction in the estimate of the error variance when they were applied to the later season resident population.

Precautions to be observed in the application of analysis of variance to data of the Poisson type are indicated.

This paper views field-plot lay-out solely from the standpoint of the distribution of insect populations, but attention is called to the fact that other considerations may be of importance.

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EFFECTS OF CERTAIN SOIL FUNGI AND THEIR BY-PRODUCTS ON OPHIOBOLUS GRAMINIS[1]

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INTRODUCTION

In studies on the take-all foot rot of wheat (*Triticum aestivum* L. (*T. vulgare* Vill.)), caused by *Ophiobolus graminis* Sacc., certain features of its development and disappearance in cultivated fields and of its control by adding organic matter and other amendments to the soil have been difficult to explain. Some workers have suggested that the development of the disease may be affected markedly by other micro-organisms in the soil.

Sanford and Broadfoot (*8*)[2] and Broadfoot (*1*) studied the effect of other organisms on the development of *Ophiobolus graminis* in pure culture and in the soil. Russell (*7*) concluded that the beneficial effect of crop rotation and fallowing on reduction of take-all was due to other soil organisms crowding out *O. graminis* in the absence of its host. Brömmelhues (*2*) reported that several fungi, when grown in artificial media, produced thermostable byproducts that inhibited the growth of *O. graminis* on solid and liquid media. She stated that a preliminary exposure of wheat to certain organisms allowed *O. graminis* to cause more damage than the simultaneous use of those fungi and *O. graminis*. The reason given is that the byproducts of the fungi tested slightly injured the plants and permitted easy infection by *O. graminis*, whereas their byproducts when they were used with *O. graminis* tended to lessen the damage from it. This is cited to explain why take-all is more severe on light than on heavy soil, as the former has less absorptive capacity. Garrett (*5*) stated that the biologic control of the parasite by other micro-organisms best explained some of the phenomena he encountered; Lal (*6*) showed that several soil fungi and bacteria are injurious to *O. graminis*, some by direct attack and others by the deleterious effects of their byproducts; and Winter (*13*) reported the presence in soil extracts of substances inhibitory to the development of *O. graminis*.

Clark (*3*) and Stumbo, Gainey, and Clark (*9*) studied the effect of organic and inorganic soil amendments on the control of take-all. They decided that the control obtained by the amendments they used was due to adequate fertilization of the soil. Their studies of soil flora were only quantitative, however, for no account was taken of the species present or of their byproducts under the circumstances imposed.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 292.

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This paper reports attempts to find some of the interrelations between the take-all fungus (*Ophiobolus graminis*) and certain other soil-inhabiting fungi and to ascertain whether variations in the severity of the disease attack by *O. graminis* can be explained on the basis of interference by certain other soil fungi. The paper is not intended as a compendium of the interrelations between a large number of soil fungi and *O. graminis*.

MATERIALS AND METHODS

The fungi studied were isolated from various types of soil used for growing wheat in central Kansas, some being free from *Ophiobolus graminis* and others infested with it. Fungi also were isolated from such infested soils after they had been treated experimentally in various ways in the field and in the greenhouse. Most of the isolations were made from the soil by the poured-plate method, but some were made from root fragments. The media employed included acid and neutral potato-dextrose agar, Lipman and Brown agar, urea agar, nutrient agar, water agar, soil-extract agar, and combinations of water and soil-extract agars.

The fungus isolates were tested for possible antibiosis or probiosis [3] to *Ophiobolus graminis* in artificial media and in the soil. Three series of tests were made. In series 1 *O. graminis* and each of the soil fungi studied were grown together on potato-dextrose agar in 100- by 15-mm. petri dishes. In series 2 the effect of byproducts of the various soil fungi on *O. graminis* was determined. In this series the fungus being tested was grown for 7 to 14 days on a potato-dextrose solution or other liquid media; then the solution was filtered through filter paper and sterilized by autoclaving at 17 pounds' pressure for 20 minutes. Different quantities of the sterile filtrate were then added to fresh, sterile potato-dextrose solution in 125-cc. Erlenmeyer flasks or to sterile, melted potato-dextrose agar in test tubes for use in petri dishes.

Later the flasks of liquid medium and the petri dishes containing the mixtures were inoculated with pieces of agar 2 mm. square cut from portions of an agar plate carrying *Ophiobolus graminis* in an actively growing condition. The flasks and petri dishes were then incubated at 22° to 26° C. The rate of growth of *O. graminis* on the agar plates was determined by measuring the diameters of the circular colonies daily until the plates were covered; it took 7 to 10 days for the colonies to cover the plates if no antibiosis or probiosis was involved. In the liquid media the dry weights of the fungus mats were taken when the experiment was terminated, usually after 14 days. Use of agar plates had the advantage of showing daily increases in growth and of requiring less time, but the use of flasks was less subject to experimental error.

In series 3 the fungi were grown together in variously treated soils which were then planted to wheat. In part of this series the wheat seedlings were grown in the laboratory in 125-cc. Erlenmeyer flasks containing 60 gm. of sterilized soil which had been inoculated with *Ophiobolus graminis* and the fungus to be tested. After *O. graminis*

[3] In the present paper antibiosis is used to mean antagonistic relations of the soil fungi or their byproducts to *Ophiobolus graminis* and probiosis to mean the opposite, that is, favorable relations to *O. graminis*.

had grown through the soil, the fungus to be tested was introduced and the two fungi were permitted to grow together for a time before germinated, surface-sterilized wheat kernels were planted in the flask. At the end of the experiment the extent and severity of root lesions on the seedlings were recorded. In part of series 3 the wheat plants were grown to maturity in the greenhouse in 6-inch clay pots containing soil naturally infested with *O. graminis* and artificially inoculated separately with the different soil fungi to be tested. Data on the extent and severity of the lesions and on the general condition of the wheat plants were taken after they had headed.

FUNGI ISOLATED

Numerous soil and root isolates were cultured and tested for the production of substances that influence the development of *Ophiobolus graminis*. The number of isolates (species or strains) in different genera was as follows: *Penicillium*, 29; *Fusarium*, 20; *Actinomyces*, 17; *Aspergillus*, 16; *Ophiobolus*, 8; *Rhizoctonia*, 7; *Gliocladium*, 6; *Pythium*, 5; *Chaetomium*, 5; *Rhizopus*, 4; *Helminthosporium*, 4; *Alternaria*, 4; *Trichoderma*, 3; *Monilia*, 3; *Trichothecium*, 1; *Acrothecium*, 1; *Spicaria*, 1; and unidentified, 17. These fungi were numbered, and the species of those that tended to be antibiotic or especially probiotic to *O. graminis* were identified whenever possible. These were the principal fungi used in the experiments.

EXPERIMENTS IN ARTIFICIAL CULTURE MEDIA

EFFECT OF OTHER SOIL FUNGI ON OPHIOBOLUS GRAMINIS

In the experiments in which *Ophiobolus graminis* was grown in the same petri dish with another fungus (series 1), potato-dextrose agar was used in duplicate plates. Twenty-seven isolates of common soil fungi were tested separately with *O. graminis*. Each of these and *O. graminis* were placed about 40 mm. apart on opposite sides of an agar plate. The fungus growth, particularly where the hyphae of the two colonies advanced toward each other, was examined several times daily.

A few of the soil fungi studied were actively antagonistic to *Ophiobolus graminis*. These were *Trichoderma lignorum* (Tode) Harz (No. 1), *Aspergillus niger* v. Tiegh. (No. 10), and *Gliocladium fimbriatum* Gilman and Abbott (No. 28). In case of these species the hyphae of *O. graminis* and those of the other fungus advanced on the agar plate until they met. Soon thereafter the hyphae of *O. graminis* died back and disintegrated where the colonies met, and the colony of the other fungus continued to advance. The hyphae of *T. lignorum* actually parasitized those of *O. graminis* by penetrating and killing them. It was not determined how the other two fungi killed the hyphae of *O. graminis*.

Ophiobolus graminis and certain other fungi showed mutual repellence. Both advancing colonies stopped growth at the inside border before the hyphae met, leaving between them a zone where there was no growth. The fungi that reacted in this way were *Aspergillus flavus* Lk. (No. 8), *A. nidulans* (Eidam) Wint. (No. 2), and *Penicillium lilacinum* Thom (No. 13).

The antibiotic or probiotic influence on *Ophiobolus graminis* of the other fungi tested could not be determined exactly by this method because either *O. graminis* grew over them or it was overgrown by them with no apparent injury to either.

EFFECT OF BYPRODUCTS OF OTHER SOIL FUNGI ON OPHIOBOLUS GRAMINIS

Many tests (series 2) were made to determine whether certain soil fungi produced substances toxic or stimulatory to *Ophiobolus graminis*. With few exceptions the medium used was the potato-dextrose solution as described previously. Soil decoctions and Czapek's solution containing various nitrogen compounds were tried also. Measured quantities of the sterile filtrate from the solutions in which individual fungi to be tested had grown (15 percent by volume) were added to a medium in which *O. graminis* was then introduced. Some fungus isolates were tested four or five times and others only once. The resulting growth was recorded as a measure of any possible toxicity. Data were taken both on the increase in diameter of the colonies of *O. graminis* on agar and on the weight of the mycelial mats produced in the liquid medium. The growth of *O. graminis* on the particular medium without the addition of any filtrate was taken as the control.

Examples of both inhibitory and stimulatory effects of various fungi in the test on potato-dextrose-agar plates are shown in figure 1. The average weights of mycelium in liquid medium (potato-dextrose solution) and the measurements of radial growth on agar for 21 of the soil-fungus isolates are shown in table 1. These isolates were selected and identified as to species because they gave a fair representation of the biotic effects encountered.

Thirty-nine, or about one-third, of the isolates included in these experiments produced substances that reduced the growth of *Ophiobolus graminis* more than 20 percent; 20 of these reduced the growth 50 percent or more; and 6 reduced it more than 80 percent. *Aspergillus niger* (No. 10) and *A. terreus* (No. 86) inhibited the growth of *O. graminis* completely.

Four of the six fungi that reduced the development of *Ophiobolus graminis* 80 percent or more were species of *Aspergillus*. According to Thom and Church (*10*) these species fall in the *A. flavus*, *A. niger*, and *A. terreus* groups and have been identified by the authors as strains of *A. flavus* (No. 8), *A. niger* (No. 10), *A. flavipes* (No. 80), and *A. terreus* (No. 86).

On the other hand, eight of the soil-fungus isolates tested in these experiments produced substances that stimulated the growth of *Ophiobolus graminis* 25 percent or more above normal.

The time of production of inhibitory growth substances in artificial media by the soil fungi tested varied considerably. Some produced appreciable quantities in 48 hours and reached their maximum production in 5 days. Others required a week or more to produce any toxic substance and 20 days to attain maximum production. *Rhizopus nigricans* produced inhibitory substances in its early stages of growth and stimulatory ones later. Some isolates of certain species of *Aspergillus*, especially *A. terreus* and *A. niger*, showed wide differences in biotic behavior toward *Ophiobolus graminis*. Such physiologic strains within a species could not always be separated morphologically.

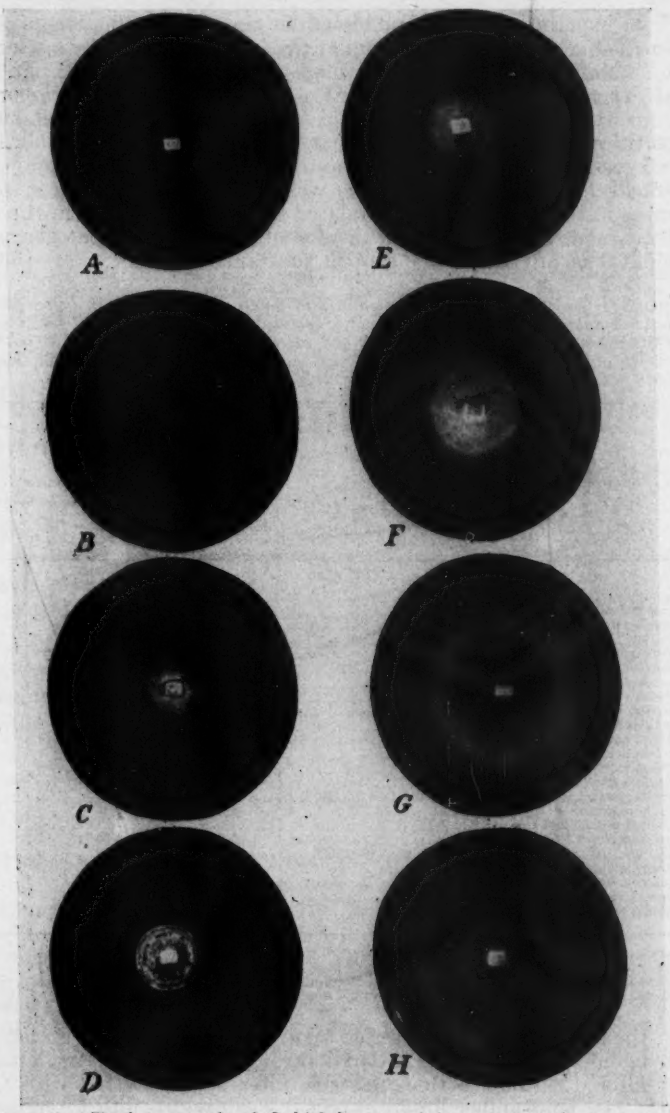

FIGURE 1.—Six-day growth of *Ophiobolus graminis* on potato-dextrose agar to which had been added 15-percent portions of sterile potato-dextrose solutions in which the soil fungi indicated had grown: *A*, *Aspergillus niger* (No. 10), no growth; *B*, *A. flavus* (No. 8), trace of growth; *C*, *Gliocladium fimbriatum* (No. 28), 21 mm. in diameter; *D*, *Trichoderma lignorum* (No. 1), 24 mm. in diameter; *E*, *Penicillium lilacinum* (No. 13), 26 mm. in diameter; *F*, *A. nidulans* (No. 2), 55 mm. in diameter; *G*, *A. terreus* (No. 3), 73 mm. in diameter; *H*, *Alternaria humicola* (No. 17), 85 mm. in diameter. The control colony of *O. graminis* without any added byproduct was 62 mm. in diameter.

Two experiments were conducted to test the production of substances affecting the development of *Ophiobolus graminis* by a selected list (table 2) of isolates of soil fungi grown in soil extract. In the first experiment, a preliminary one, soil extracts that had supported the growth of the various soil fungi to be tested were added in small quantities to potato-dextrose agar and potato-dextrose solution; in all cases the growth of *O. graminis* on these media was stimulated markedly. This indicated either that these fungi produced substances in soil extract that stimulated *O. graminis* or that in the soil extract itself there was a growth-promoting substance that obscured the effect of any inhibitory growth substance formed.

TABLE 1.—*Growth of Ophiobolus graminis in potato-dextrose solution or on potato-dextrose agar to which had been added 15-percent portions of sterilized solutions in which various individual soil fungi had grown previously as compared with its growth on the same medium without such addition (control), Manhattan, Kans., 1938*

Fungus grown in solution that was added	Growth of *O. graminis*				
	Weight of fungus mats in solution	Diameter on agar	Compared with growth of control		
			In solution	On agar [1]	Average
	Gm.	*Mm.*	*Pct.*	*Pct.*	*Pct.*
Actinomyces sp. (No. 2A)	0.0574	82	95	117	106
Alternaria humicola Oudemans (No. 17)	.0670	77	111	110	111
Aspergillus flavipes (Bainer and Sartory) Thom and Church (No. 80)	.0089	0	15	0	8
Aspergillus flavus (No. 8)	.0082	25	14	36	25
Aspergillus nidulans (No. 2)	.0553	57	92	81	86
Aspergillus niger (No. 10) [2]	.0000	0	0	0	0
Aspergillus niger (No. 47) [2]	.1082	70	180	100	140
Aspergillus terreus Thom (No. 3) [3]	.0674	77	112	110	111
Aspergillus terreus (No. 86) [3]	.0000	0	0	0	0
Fusarium moniliforme Sheldon (No. 31) [4]	.0968	61	161	87	124
Fusarium moniliforme (No. 109) [4]	.0000	51	0	73	36
Fusarium moniliforme (No. 14) [4]	.0618	69	103	99	101
Gliocladium fimbriatum (No. 28)	.0000	25	0	36	18
Helminthosporium sativum Pam., King, and Bakke (No. 27)	.0667	88	111	126	118
Penicillium lilacinum (No. 13)	.0269	27	45	39	42
Penicillium pinophilum Hedgc. (No. 98)	.0130	25	22	36	29
Rhizopus nigricans Ehr. ex Fr. (No. 4)	.0206	27	34	39	37
Spicaria divaricata (Thom) Gilman and Abbott (No. 116)	.0063	0	10	0	5
Trichoderma lignorum (No. 1)	.0038	37	6	53	30
Trichoderma lignorum (No. 34)	.0066	36	11	51	31
Trichothecium roseum Lk. (No. 29)	.0643	70	107	100	104
Ophiobolus graminis (control)	.0601	70	100	100	100

[1] Percentage computed by comparison of diameters.
[2] These 2 isolates of *Aspergillus niger* could not be separated morphologically.
[3] These 2 isolates of *A. terreus* could not be separated morphologically.
[4] These 3 isolates of *Fusarium moniliforme* could not be separated morphologically.

In the second soil-extract series 5 kg. of garden soil was extracted with boiling distilled water, filtered through cheesecloth, and finally force-filtered through 6 thicknesses of filter paper covered with fine mud. The light-amber liquid obtained was divided into 11 (60-cc.) aliquots in Erlenmeyer flasks and was sterilized 1 hour in the autoclave at 15 pounds' pressure on three successive days. The flasks were then inoculated separately with the following soil fungi: *Alternaria humicola*, *Aspergillus flavus*, *A. niger*, *A. terreus*, *Gliocladium fimbriatum*, *Penicillium lilacinum*, *P. viridicatum* Westling, *Penicillium* sp. (No. 21), *Rhizopus nigricans*, *Trichoderma lignorum*, and *Ophiobo-*

lus graminis. Growth was slow and sparse. After 60 days all flasks were sterilized again by autoclaving, their contents were filtered, and the filtrates were used as 15- and 25-percent (by volume) additions to potato-dextrose agar and potato-dextrose solution in petri dishes and flasks as previously described. *O. graminis* was then grown in each of these. Data on the growth of *O. graminis* in the petri dishes and the flasks were so similar that only those from the flasks are given in table 2.

TABLE 2.—*Growth of Ophiobolus graminis in potato-dextrose solution to which had been added 15- or 25-percent portions of sterilized soil extracts in which various individual soil fungi had grown previously as compared with its growth in the same medium without such addition (control), Manhattan, Kans., 1938*

Fungus grown in solution that was added	Soil extract added	Growth of *O. graminis*	
		Weight of fungus mats	Compared with growth of control
	Percent	*Grams*	*Percent*
Alternaria humicola (No. 17)	15	0.1224	139
	25	.1416	158
Aspergillus flavus (No. 8)	15	.1205	135
	25	.1068	119
Aspergillus niger (No. 10)	15	.1320	147
	25	.1050	117
Aspergillus terreus (No. 3)	15	.1102	123
	25	.1029	115
Gliocladium fimbriatum (No. 28)	15	.1021	114
	25	.0923	103
Penicillium lilacinum (No. 13)	15	.1196	134
	25	.1150	128
Penicillium viridicatum (No. 26)	15	.0999	112
	25	.1430	160
Penicillium sp. (No. 21)	15	.1526	170
	25	.1268	142
Rhizopus nigricans (No. 4)	15	.1047	117
	25	.1492	167
Trichoderma lignorum (No. 34)	15	.1076	120
	25	.1123	125
Ophiobolus graminis	15	.0984	110
	25	.0976	109
Ophiobolus graminis (control)	0	[1] .0895	100

[1] Average of 4 controls.

It is apparent from table 2 that the soil extract which had served as a medium stimulated the growth of *Ophiobolus graminis* in every instance. It is also apparent in the case of *Aspergillus terreus*, *A. flavus*, *A. niger*, *Penicillium* sp. (No. 21), *Gliocladium fimbriatum*, and *Penicillium lilacinum* that the 25-percent addition produced less growth than the 15-percent addition. It might be assumed that the greater amounts of inhibitory material present in the 25-percent addition were responsible for the reduced growth. These particular isolates had been found to be toxic in previous tests in other media, whereas, of the four fungi that exhibited greater growth after the 25-percent addition than after the 15-percent, *Rhizopus nigricans* and *Alternaria humicola* had often produced growth materials stimulatory to *O. graminis* in previous experiments.

Experiments were performed to study the production of byproducts inhibitory to *Ophiobolus graminis* by soil fungi nourished by different sources of nitrogen. Four lots of Czapek's solution were made up, each carrying a different nitrogen compound in concentrations of 2 gm. of the compound per liter. The nitrogen sources used were

ammonium chloride, ammonium nitrate, sodium nitrate, and urea. Potato-dextrose solution was included for comparison. Fifty cubic centimeters of each nutrient solution was placed in 125-cc. Erlenmeyer flasks, plugged with cotton, and sterilized in the autoclave for 1 hour at 15 pounds' pressure. These were then inoculated separately with seven selected soil isolates, namely *Aspergillus flavus*, *A. nidulans*, *A. niger*, *Gliocladium fimbriatum*, *Penicillium lilacinum*, *Rhizopus nigricans*, and *Trichoderma lignorum*. All except *A. nidulans* and *R. nigricans* had been fairly uniform in producing in potato-dextrose solution substances that inhibited *O. graminis*. Each fungus was grown in each of the five solutions for 10 days. All grew except *R. nigricans* with sodium nitrate and *T. lignorum* with ammonium chloride as sources of nitrogen.

After 10 days the solutions were filtered, sterilized, and mixed with potato-dextrose agar or solution so that the fungus extract would constitute 15 percent (by volume) of the mixture. The mixtures were placed in petri dishes and flasks, respectively. These were then inoculated with *Ophiobolus graminis*. As a control *O. graminis* was grown in potato-dextrose solution without the addition of nitrogen compounds and without filtrates containing byproducts of other fungi. The weights of fungus mats from the flasks, which represent the comparative growth, are shown in table 3.

TABLE 3.—*Growth of Ophiobolus graminis in potato-dextrose solution to which had been added 15-percent portions of sterilized solutions in which various individual soil fungi previously had been nourished by different sources of nitrogen as compared with its growth in the same medium without such addition (control), Manhattan, Kans., 1938*

Nitrogen source or medium and fungus grown in solution that was added	Growth of *O. graminis*		Nitrogen source or medium and fungus grown in solution that was added	Growth of *O. graminis*	
	Weight of fungus mats [1]	Compared with growth of control [2]		Weight of fungus mats [1]	Compared with growth of control [2]
Ammonium chloride:	*Grams*	*Percent*	Urea:	*Grams*	*Percent*
Aspergillus niger (No. 10)	0.0000	0	*Gliocladium fimbriatum* (No. 28)	0.0000	0
Aspergillus flavus (No. 8)	.0207	33	*Penicillium lilacinum* (No. 13)	.0523	84
Rhizopus nigricans (No. 4)	.0468	76	*Aspergillus niger* (No. 10)	.0550	89
Penicillium lilacinum (No. 13)	.0501	81	*Aspergillus flavus* (No. 8)	.0594	96
Gliocladium fimbriatum (No. 28)	.0522	84	*Aspergillus nidulans* (No. 2)	.0664	107
Aspergillus nidulans (No. 2)	.0613	99	*Trichoderma lignorum* (No. 1)	.0718	116
Ammonium nitrate:			*Rhizopus nigricans* (No. 4)	.0849	137
Gliocladium fimbriatum (No. 28)	.0000	0	Potato-dextrose solution:		
Aspergillus nidulans (No. 2)	.0524	85	*Aspergillus niger* (No. 10)	.0000	0
Aspergillus niger (No. 10)	.0585	94	*Rhizopus nigricans* (No. 4)	.0072	12
Trichoderma lignorum (No. 1)	.0633	102	*Aspergillus flavus* (No. 8)	.0098	16
Penicillium lilacinum (No. 13)	.0688	111	*Penicillium lilacinum* (No. 13)	.0493	80
Aspergillus flavus (No. 8)	.0942	152	*Gliocladium fimbriatum* (No. 28)	.0568	92
Rhizopus nigricans (No. 4)	.1500	242	*Aspergillus nidulans* (No. 2)	.0603	97
Sodium nitrate:			*Trichoderma lignorum* (No. 1)	.0614	99
Aspergillus niger (No. 10)	.0000	0	Control (potato-dextrose solution alone):		
Aspergillus flavus (No. 8)	.0305	49	*Ophiobolus graminis*	.0620	100
Gliocladium fimbriatum (No. 28)	.0525	85			
Penicillium lilacinum (No. 13)	.0587	95			
Trichoderma lignorum (No. 1)	.0597	96			
Aspergillus nidulans (No. 2)	.0810	131			

[1] Average of 2 replicates.

[2] Fungi listed in each group in descending order of inhibitory action on *Ophiobolus graminis*.

It will be seen in table 3 that the growth of *Ophiobolus graminis* may be variously affected by the byproducts from fungi grown in media with different nitrogen sources. These effects ranged from complete inhibition of *O. graminis* to striking stimulation.

Aspergillus niger grown in Czapek's solution with ammonium chloride or sodium nitrate or in potato-dextrose solution developed byproducts that completely inhibited *Ophiobolus graminis;* only slight inhibition resulted when it was grown with ammonium nitrate or urea. In contrast, *Gliocladium fimbriatum* developed byproducts that effected complete inhibition of *O. graminis* when ammonium nitrate or urea was the nitrogen source but not when the other compounds were used.

Aspergillus flavus when grown in Czapek's solution with ammonium chloride or sodium nitrate or in potato-dextrose solution developed byproducts that inhibited but did not prevent growth of *Ophiobolus graminis.* This is in agreement with the results shown in table 1. In general, the byproducts from *A. flavus* seemed to be of the same nature as those from *A. niger,* but the inhibitory effects were less. It is of special interest to note that the byproducts from *A. flavus* grown with ammonium nitrate were distinctly stimulatory to *O. graminis,* producing 152 percent as much growth as the control.

Rhizopus nigricans developed inhibitory byproducts when grown in potato-dextrose solution or in Czapek's solution with ammonium chloride as the nitrogen source. When ammonium nitrate or urea was used, its byproducts were distinctly stimulatory, causing 242 and 137 percent growth of *Ophiobolus graminis,* respectively.

The byproducts of *Penicillium lilacinum, Aspergillus nidulans,* and *Trichoderma lignorum* were only slightly inhibitory, neutral, or slightly stimulatory. The byproducts from these three fungi also had somewhat similar effects in the earlier series, but the inhibition was greater (table 1).

The unused nitrogen remaining in the solutions in which the soil fungi had grown was not responsible for growth stimulation, as Fellows (*4*) has shown previously that *Ophiobolus graminis* cannot utilize any of the tested sources of nitrogen in a modified Czapek's solution. It is not believed that any of the other unused compounds of Czapek's solution were stimulatory, since *O. graminis* grows better in potato-dextrose solution alone than in modified Czapek's solution even when there is a source of nitrogen in the latter that is favorable for its growth.

The experiment indicates that the production by other soil fungi of substances that are injurious or beneficial to *Ophiobolus graminis* depends on the nature of the soil nutrients as well as on the kinds of fungi present. It indicates that some soil fungi may be distinctly beneficial to *O. graminis* under certain nutritional conditions and decidedly injurious under other conditions. It also suggests that the inhibitory substances produced by one fungus may be different from those produced by another, since they apparently are formed from different materials.

EXPERIMENTS IN SOIL

The interaction of *Ophiobolus graminis* and certain soil fungi was tested both in sterilized, artificially infested soil and in unsterilized, naturally infested soil (series 3). A method was devised for testing

the reaction of *O. graminis* to any other soil fungus by determining the effect of the latter on the pathogenicity of *O. graminis* to wheat seedlings and older wheat plants. In order to avoid introduction of undesirable organic matter, both *O. graminis* and the other soil fungi used in these experiments were cultured in the soil.

EXPERIMENTS IN STERILIZED SOIL

All experiments with sterile soil were in 125-cc. Erlenmeyer flasks containing 60 gm. of soil moistened to approximately 70 percent of its water-holding capacity and steam-sterilized in the flasks. Except in the uninoculated controls the soil was then inoculated with *Ophiobolus graminis* by introducing a 2-mm. cube of agar upon which the fungus was growing. After 14 days' growth of *O. graminis* the soil in some of the flasks was inoculated similarly with the other fungus to be tested. Other flasks were left as inoculated controls, that is, they contained only *O. graminis*. Fourteen days later a surface-sterile wheat seedling was planted in the soil in each flask and allowed to grow 14 days. At that time *O. graminis* had been in the soil 42 days, the other fungus 28 days, and the wheat seedling 14 days. During all this time the inoculated soil and the wheat plant were kept as free as possible from external contamination. The lengths of the three primary roots and of the diseased portions were then measured, and the percentage of root length showing disease lesions was calculated. Twenty-three fungi were tested by this method. Two virulent and one moderately pathogenic strain of *O. graminis* were used. It was found that any fungus that checked the pathogenicity of *O. graminis* checked the moderately pathogenic strain more effectively than it did the highly virulent ones but in the same order. In one experiment seven fungi were used with a moderately pathogenic strain of *O. graminis*. The results are given in table 4.

TABLE 4.—*Effect of certain soil fungi on the growth of wheat roots and on take-all in soil sterilized and then inoculated with Ophiobolus graminis, Manhattan, Kans., 1939*

Fungus added to soil with *Ophiobolus graminis*	Average length of 3 primary roots	Average root length diseased		General appearance and remarks
	Mm.	*Mm.*	*Percent*	
Aspergillus flavus (No. 8)	125	32	26	Fair control.
Aspergillus niger (No. 10)	130	68	52	Good control in 1 flask; none in others.
Fusarium moniliforme (No. 31)	121	22	18	Good control in 2 flask; fair control in 1.
Gliocladium fimbriatum (No. 28)	110	57	52	Some control.
Ophiobolus graminis (control)	117	86	74	Badly diseased.
Pythium arrhenomanes (No. 126)	58	41	70	No control; badly diseased.
Rhizopus nigricans (No. 4)	106	36	34	Doubtful; contaminated with No. 1.
Trichoderma lignorum (No. 1)	114	3	3	Good control.

As shown in table 4, fair to good control of *Ophiobolus graminis* was obtained by artificially inoculating infested soil with *Trichoderma lignorum* (No. 1), *Aspergillus flavus* (No. 8), or *Fusarium moniliforme* (No. 31). Less control was obtained with *A. niger* (No. 10) and *Gliocladium fimbriatum* (No. 28) and none with *Pythium arrhenomanes* Drechs. (No. 126), which is known to be parasitic on wheat. The

flasks containing *Rhizopus nigricans* (No. 4) were accidentally contaminated with *Trichoderma lignorum* (No. 1), obscuring possible effects of the former.

In the uninoculated, sterilized soil the wheat roots and tops were stunted and short but the roots were white and clean. Seemingly there was some toxic effect of steam sterilization of the soil, as the root lengths averaged only 40 mm. as compared with 100 and 150 mm. for those in soils to which fungi had been added. Most of the fungi used, including *Ophiobolus graminis*, dissipated this inhibitory effect rapidly when cultured in the sterilized soil. An exception was *Rhizopus nigricans* (No. 4) in the presence of which the wheat roots were similar to those in uninoculated, sterilized soil. The reason may have been that this fungus inhibited root growth, or it may not have dissipated the injurious effect of steam sterilization, as did the others.

EXPERIMENTS IN NATURALLY INFESTED SOIL

Experiments in which various soil fungi were introduced into soil naturally infested with *Ophiobolus graminis* were rather limited. However, they show that the severity of take-all was reduced by increasing the population of certain species in a soil containing its natural flora.

In one experiment the fungi to be tested were cultured separately under aseptic conditions in moist, sterilized soil in 1-liter Erlenmeyer flasks. Inoculation was accomplished by a 2-mm. block of agar upon which the fungus to be tested was growing. After sufficient growth had occurred, as judged by the penetration through the soil, the culture was mixed with soil naturally infested with *Ophiobolus graminis* at the rate of 1 part by volume of inoculated soil to 3 parts of naturally infested soil. The mixture was placed in 6-inch pots in the greenhouse, and Kanred wheat was planted in it. For the fungi that formed spores, this inoculum was supplemented by adding spores from a petri-dish culture to the top 3 inches of the soil. Planting was done after inoculation. The final examination was made when the wheat plants were nearly mature. Two kinds of controls were used; one consisted of a mixture of 3 parts of naturally infested soil and 1 part sterilized soil and the other of sterilized soil alone.

The effect on the severity of take-all of adding *Aspergillus flavus* (No. 8) to naturally infested and to artificially inoculated soil is shown in figure 2. The seedling roots shown in *A* and *B* grew in soil that had been artificially inoculated with a pure culture of *Ophiobolus graminis*, but the roots in *B* had been protected by *A. flavus* (No. 8), which was added to the soil before the wheat was planted. As shown in table 4, the average root length infected in *A* was 74 percent, whereas that in *B* was only 26 percent.

The control wheat plants, which were almost entirely killed, were grown in soil naturally infested with *Ophiobolus graminis* (fig. 2, *C*). The plants shown in figure 2, *D*, were grown on part of the same lot of naturally infested soil that had been inoculated with *Aspergillus flavus* (No. 8) before the wheat was planted. Although there was some infection, a fair growth of wheat occurred.

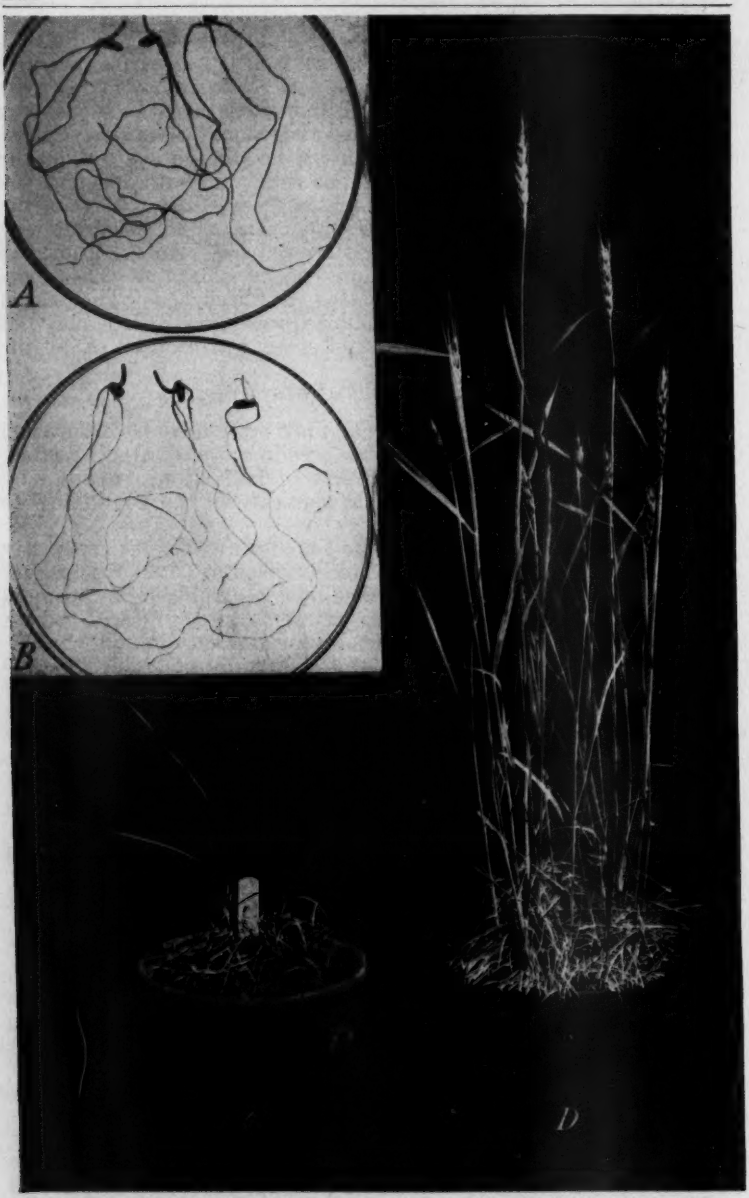

FIGURE 2.—*A* and *B*, Roots of wheat seedlings grown in soil artificially inoculated with *Ophiobolus graminis: A*, *O. graminis* alone, 74 percent of root length infected; *B*, with *Aspergillus flavus* (No. 8) added to the soil, 26 percent of length infected. *C*, Wheat plants, almost entirely killed by take-all, grown in soil naturally infested with *O. graminis*. *D*, Wheat plants grown in part of the same soil as *C* with *A. flavus* (No. 8) added to it before the wheat was sown.

DISCUSSION

The data presented herein serve mainly to emphasize the complexities of the problems involved rather than to give any solution. The interrelations of soil fungi, especially when one or more are pathogenic, seem of sufficient importance to merit study, particularly as there are still many unexplained phenomena.

In the present study a number of common soil fungi were found to be antagonistic to *Ophiobolus graminis* in one or more ways and some were stimulatory. Some fungi, *Trichoderma lignorum*, for example, seemed capable of direct parasitism; others under certain conditions produced growth substances distinctly injurious to *O. graminis;* and still others produced byproducts markedly stimulatory to *O. graminis*. Some were capable of either injury or stimulation, depending on the particular set of conditions under which they were grown. Under a given set of conditions, one soil fungus was distinctly antagonistic to *O. graminis* whereas another was beneficial. Under another set of conditions the effects were reversed. In the light of this information, it may be concluded that the applications of certain soil amendments may enable some very common soil fungi to produce byproducts either antibiotic or probiotic to *O. graminis*.

Under the conditions of the experiments at least one fungus (*Rhizopus nigricans*) produced toxic substances during its early growth and markedly stimulatory substances later.

It seems entirely probable that there are several different byproducts of fungus growth capable of inhibiting development of *Ophiobolus graminis*, as such substances are produced from different materials and by different fungi. Furthermore, the production of the byproducts is affected differently by certain physical changes.

The fragmentary evidence obtained in these studies indicates that the metabolism of the soil fungi studied differs widely. For example, *Rhizopus nigricans* was unable to grow in Czapek's solution with sodium nitrate as a source of nitrogen, but it made satisfactory growth when the nitrogen was from other sources. In the same experiment, *Trichoderma lignorum* was unable to grow in Czapek's solution with ammonium chloride as a nitrogen source but it made a normal growth when other compounds were the sources of nitrogen.

It may be noted also that *Gliocladium fimbriatum* and *Rhizopus nigricans* reversed their positions in respect to production of inhibitory byproducts in potato-dextrose solution in the experiments reported in tables 1 and 3. In partial explanation of this seeming discrepancy it may be stated that the 10-day period of growth used in the latter experiment was probably too short for maximum production of inhibitory substances from potato-dextrose media by *G. fimbriatum*. This fungus, which grows rather slowly, required a 20-day growth period for maximum production of toxic substances in potato-dextrose solution, whereas *R. nigricans*, which grows rapidly, required only 5 days to reach maximum production of the inhibitory substances in the same medium. After 15 and 20 days' growth in this medium, however, it was found in several tests to be producing stimulatory byproducts.

The marked stimulation of the growth of *Ophiobolus graminis* caused by byproducts of *Rhizopus nigricans* grown in Czapek's solution with ammonium nitrate as a source of nitrogen is of much

interest. The small quantity of growth solution added could not have furnished any large amount of food. Stimulation must have been in the nature of a hormone, or a "bios." Soil fungi parasitic or inhibitory to other soil fungi have been reported by Weindling (*11*), who also wrote an extensive treatise on association effects of fungi (*12*).

In artificially inoculated soil the parasitism of *Ophiobolus graminis* on wheat roots was definitely lessened by the presence of several other common soil fungi, as shown in table 4. Also in naturally infested field soil the severity of take-all usually was lessened by increasing the population of certain fungi, but the results were less consistent.

SUMMARY

Fungi capable of producing substances that inhibit the growth of *Ophiobolus graminis* in pure culture and of preventing or lessening infection of wheat by *O. graminis* in soil were isolated from soils in central Kansas, both those in which *O. graminis* occurred and those in which it did not occur.

Fungi, the byproducts of which stimulated *Ophiobolus graminis* in pure culture, also were isolated.

Among 143 species and strains of fungi tested, about 25 percent produced in pure culture byproducts that were inhibitory to *Ophiobolus graminis* and about 10 percent produced those that were stimulatory.

The production of inhibitory or stimulatory byproducts by any fungus varied with its stage of growth and the nature of the substrate on which it was cultured. Some fungi produced inhibitory byproducts on one culture medium and stimulatory byproducts on another. On the same substrate inhibitory byproducts were produced by one fungus and stimulatory substances by another.

Several soil fungi decreased the pathogenicity of *Ophiobolus graminis* on wheat in both artificially inoculated and naturally infested soil. The degree of inhibitory action in soil shown by any fungus toward *O. graminis* varied somewhat with the strain of the latter.

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THE EFFECT OF SHORT PHOTOPERIOD ON SORGHUM VARIETIES AND FIRST GENERATION HYBRIDS [1]

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INTRODUCTION

There are numerous varieties of sorghum (*Sorghum vulgare* Pers.) grown in the United States, a score or more of which have been introduced and 40 or 50 of which have been produced through hybridization and selection. In Africa and Asia, where the species is indigenous, there are literally hundreds of varieties. As would be expected, the varietal differences are of many kinds and include contrasting characters involving glume color and texture, awns, of pericarp chloroplast, and other plant colors, types of endosperm starch, integuments, juiciness and sweetness of stem, plant height, rate of tillering, and duration of growth. Many of the differentiating characteristics, including duration of growth, have been shown to be under genetic control. Differences in adaptation other than those influenced by the known genes that determine plant response to photoperiod are also assumed to be genetic. It would be useful to know how sorghum varieties respond to changes in photoperiod since such knowledge would furnish a basis for a genetic classification.

REVIEW OF LITERATURE

The literature concerning the reactions of plants to photoperiod, temperature, and nutrition, and the differential response of varieties to identical treatments has become extensive. It is now generally accepted as a fact that differences in maturity among varieties of many species are brought about by different reactions to environment.

Thompson (*8*) [2] has reviewed the literature that shows the relation between temperature and vegetative and reproductive development in plants. The work in this field shows that the prevailing temperature may determine whether or not a plant will be photoperiodically sensitive. According to Hamner (*3*), most investigators have concluded that if a species contains any strains that can be classed without question in either the long-day or the short-day group, then all other strains of the same species will tend to exhibit responses which would place them in the same group. The varieties or strains of such a species may be arranged in a graded series according to photoperiodic response with the sensitive strains at one end and the more or less day-neutral strains at the other. The inference from this work is that the thermal requirements have not been met whenever a variety or

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[2] Italic numbers in parentheses refer to Literature Cited, p. 300.

strain of a photoperiodically sensitive species does not respond to short (or long) photoperiods.

Just how photoperiodism fits, if it does, into the theory of phasic development is not as yet well understood, as has been pointed out by Hamner (*3*). The concept of phasic development emphasizes the fixed sequence of the phases and the differences between the thermo-scoto-, and photo-phases of plant growth. Contrary to this conception, some evidence indicates that in certain short-day species photoperiodic induction occurs during both the light and dark phases of a cycle. Also, some plants which require a treatment with low temperature have not been reported as behaving like typical short-day plants after low temperature treatment.

Garner and Allard (*2*) showed that sorghum is a short-day species. Borthwick and Parker (*1*) found that a number of soybeans varieties, all of which are sensitive to photoperiod, have different critical photoperiods. Quinby and Karper (*5*) reported that all strains of milo are sensitive to photoperiod and that the strains which have unlike durations of growth apparently differ from one another in having different critical photoperiods.

Sorghum varieties and first generation hybrids between them vary greatly in duration of growth as well as in size and grain production. Karper and Quinby (*4*) have presented data in detail on several varieties and hybrids. The lateness of maturity exhibited by certain hybrids, exclusive of the effects due to heterosis that are discussed by Quinby and Karper (*6*), is considered to be due to the action of complementary genes and it appears that the gene *Ma* is involved wherever extreme lateness occurs.

MATERIALS AND METHODS

When work on the inheritance of genes that affect maturity in sorghum was begun at the Texas station in 1938, it soon became apparent that sorghum varieties differed not only in having different critical photoperiods but also in sensitivity to short-day treatment. To investigate this point further, 12 varieties were grown in a July 3 planting in the field in 1941 at the Chillicothe substation, and part of each variety was subjected to a 10-hour photoperiod and the remainder left without treatment. A similar planting of 14 varieties and 21 first generation hybrids was made on June 30, 1942. During July at this latitude of 34° the sun is above the horizon for slightly over 14 hours. The average minimum (night) temperatures at Chillicothe during July in 1941 and 1942 were, respectively, 71.4° and 70.5° F. The corresponding maximum (day) temperatures were 95.0° and 99.9°.

The plants subjected to 10-hour photoperiods were covered, from the day of planting until after head differentiation of all varieties, from 5 p. m. to 7 a. m. with boxes covered first with rubberized cloth and then with white sheeting. The boxes were constructed in such a way as to allow ventilation. The length of the short-day treatment was 39 days in 1941 and 29 days in 1942. The plantings consisted of short rows about 8 inches apart. Seeds were planted thickly in order that there might be several plants for examination as seedlings and at stages of growth prior to the time of floral initiation and

still leave four plants to grow to maturity. After the time of floral initiation the stand of each variety was reduced to four plants in both treated and untreated plots. In a number of cases there was not sufficient crossed seed to furnish enough plants for examination in the early period of growth, which accounts for the blanks in the tabulated data. When stands were obtained as desired, four plants were present at maturity in a 15-inch row. The stands in the treated and untreated areas, each of which occupied about 45 square feet in 1942, were as nearly identical as possible.

These small areas, which consisted of fertile soil, were watered several times by flooding. Close spacing and ample moisture such as were used in this experiment apparently have no great influence as a part of the environment, since the time of anthesis of the plants under normal day length appears to be within the normal range for the various varieties. Plant size, however, was greatly reduced from that of plants grown in 40-inch rows and 8- to 12-inch spacing. The small plants in the close spacings had the same leaf number as those in wide rows. It appears that close spacing itself does not affect the time that is consumed in laying down an internode but it does affect the size of the growing point which controls the size of the leaves, culm, panicle, etc. Close spacing also retards tiller development.

The fifth and tenth leaves were permanently identified by small tags placed around the stem above the leaf. Each plant was tagged on the day of first anthesis.

The figures that appear in tables 1 and 2 were obtained in the following manner. Plants were examined for floral initiation each day after the twentieth. The first day on which a florally inducted head was found was designated as the day of head differentiation. The figures for days to first antesis and number of leaves were taken from the plant that was selected arbitrarily as being most representative of the four that grew to maturity. To determine whether floral initiation had taken place, plants were split with a pocket knife and the growing points examined under low magnification.

EXPERIMENTAL RESULTS

Data on number of days from planting to head differentiation, first anthesis, and number of leaves of the varieties and hybrids under both normal and 10-hour photoperiods are presented in tables 1 and 2.

It is quite evident that there are differences in sensitivity to 10-hour photoperiods. In 1941 the sensitive varieties differentiated their heads on the twenty-third day and in 1942 on the twenty-first to twenty-third day. This sensitive group of varieties consisted of both milos, Spur and FC 811 feteritas, both hegaris, Freed, California White durra, both kalos, Bonita, Bonar durra, and shallu. Manko was a little less sensitive to 10-hour photoperiods than the group just mentioned but was more sensitive than Blackhull kafir, Sumac, Bishop, and Lemon Yellow. Dwarf broomcorn was the one variety that failed to be affected by 10-hour photoperiods in either year. However, Texas Blackhull kafir was unaffected in 1942, the only year in which it was grown.

The number of days to first anthesis and leaf number are a reflection of the relative time at which head differentiation took place.

TABLE 1.—*Effect of 10-hour photoperiod on time of floral initiation, leaf number, and time of anthesis of sorghum varieties planted July 3, 1941, at Chillicothe, Tex.*

Serial No.	Variety	Number of days from planting to—				Number of leaves on mature plant	
		Head differentiation		First anthesis			
		Short day	Normal day	Short day	Normal day	Short day	Normal day
SA 5043	Sooner milo	23	32	43	49	11	13
TS 25243–276	Texas milo	23	39	47	68	11	18
SPI 34911	Hegari	23	48	47	77	13	18
FC 16207	Kalo	23	39	47	64	11	17
SA 208	California White durra	23	34	51	55	13	14
FC 6601	Spur feterita	23	36	56	65	16	19
SPI 29166	Freed	23	32	46	51	10	12
FC 8991	Manko	25	47	50	77	12	17
FC 8993	Bishop	28	39	61	71	14	17
SPI 35038	Sumac	29	39	60	65	14	16
FC 6607	Blackhull kafir	29	39	59	69	14	16
SPI 30204	Dwarf broomcorn	39	39	68	68	15	15

TABLE 2.—*Effect of 10-hour photoperiod on time of floral initiation leaf number, and time of anthesis of sorghum varieties planted June 30, 1942, at Chillicothe, Tex.*

Serial No.	Variety or hybrid	Number of days from planting to—				Number of leaves on mature plant	
		Head differentiation		First anthesis			
		Short day	Normal day	Short day	Normal day	Short day	Normal day
FC 8975	Sooner milo	22	27	49	49	11	15
TS 25243–276	Texas milo	22	30	46	65	13	17
SA 281	Early hegari	21	30	44	71	11	13
SPI 34911	Hegari	22	------	46	78	12	15
FC 16214	Early kalo	21	27	46	53	8	12
FC 16207	Kalo	22	29	46	60	11	14
FC 811	Feterita	21	27	45	49	11	15
FC 6601	Spur feterita	23	27	54	62	14	15
SPI 55128	Bonar durra	22	27	51	51	13	15
SA 79	Bonita	22	27	45	51	13	16
Agros. 2650	Shallu	------	------	54	75	16	18
TS 24929	Lemon Yellow	27	70	55	110	13	22
FC 8962	Texas Blackhull kafir	29	29	60	60	14	14
SPI 30204	Dwarf broomcorn	29	29	67	64	13	14
SA 1577	Texas Blackhull kafir x Sooner milo	23	25	52	52	12	16
SA 1578	Texas Blackhull kafir x Texas milo	23	------	52	79	14	22
SA 1594	Bonita x hegari	24	------	50	80	13	22
SA 1595	Bonita x Early hegari	22	------	46	79	14	24
SA 1596	Early hegari x Sooner milo	21	------	41	83	11	20
SA 1598	Hegari x Sooner milo	21	------	42	80	11	17
SA 1597	Early hegarf x Texas milo	22	------	43	80	13	23
SA 1599	Hegari x Texas milo	22	------	45	82	12	23
SA 1600	Spur feterita x Sooner milo	21	27	46	52	13	16
SA 1602	Feterita 811 x Sooner milo	22	26	43	45	13	15
SA 1601	Spur feterita x Texas milo	22	------	48	78	13	22
SA 1605	Early kalo x Sooner milo	22	27	48	51	12	15
SA 1607	Kalo x Sooner milo	22	27	44	49	11	14
SA 1608	Kalo x Texas milo	22	31	44	67	12	16
SA 1593	Bonar durra x Texas milo	22	------	43	80	12	22
SA 1585	Texas Blackhull kafir x hegari	------	------	52	100	13	21
SA 1587	Texas Blackhull kafir x kalo	23	27	46	52	12	14
SA 1588	Texas Blackhull kafir x Maizola	27	27	52	58	14	15
SA 1589	Texas Blackhull kafir x Manko	27	27	53	56	14	15
SA 1591	Texas Blackhull kafir x Bishop	27	28	55	58	14	18
SA 1582	Texas Blackhull kafir x feretita 811	22	25	44	52	13	16

The data, while conclusive, do not appear to be entirely consistent in every particular. Some deviation should be expected since the figures on head differentiation were obtained from plants that were destroyed and the figures on anthesis and leaf number from other plants that grew to maturity. However, the larger number of leaves produced by feterita than by other varieties in the same length of time is typical, as was shown by Sieglinger (*7*).

Every first generation hybrid differentiated its head along with its sensitive parent. The characteristic of being sensitive to short photoperiod, therefore, acts as a dominant. The only hybrids that approached the nonsensitive parent in lateness of head differentiation were those with two nonsensitive parents.

DISCUSSION

It is assumed that sensitivity to photoperiod indicates that the thermal requirements of the variety have been met. Likewise, it is assumed that the various degrees of insensitivity indicate that the thermal requirements have been met only partially or perhaps not at all. It It was with this idea of sensitivity to photoperiod in mind that the sorghum varieties included in this study were classified into sensitive and nonsensitive varieties. A variety is considered to be sensitive, therefore, if, under 10-hour photoperiods, it differentiates its head, or is florally inducted, in about 21 to 23 days. It is realized that the number of days would vary slightly if elements of the environment other than photoperiod are variable.

Since Texas milo is a later maturing variety than Sooner milo, administering 10-hour photoperiods reduces the time to first anthesis by approximately 20 and 6 days respectively. This difference is not considered to be due to a difference in sensitivity but comes about as a result of the fact that Texas milo and Sooner milo have different critical photoperiods. If this assumption is true, critical photoperiod and sensitivity to photoperiod are distinct manifestations. The differences in maturity and adaptation that characterize the early and late maturing strains of a variety and that result from differences in critical photoperiod are reasonably well understood, but the differences in maturity and adaptation between sorghum varieties that result from differences in sensitivity to photoperiod are not well understood. Differences due to unlike critical photoperiods are such as those between Texas and Sooner milos or between Kalo and Early Kalo; differences due to unlike sensitivity to photoperiod are such as those between Sooner milo and Blackhull kafir. Differences in critical photoperiod differentiate between strains of a single variety, whereas differences in sensitivity differentiate between varieties. The three *Ma* genes whose inheritance has been worked out in milo (*5*) all influence the critical photoperiod. The inheritance of a gene or genes that influence sensitivity to photoperiod has not been reported, but the differences in inheritance are not simple as in the case of genes that influence critical photoperiod.

It was observed in this and previous studies that once a head is initiated, the size of plant or time to anthesis was fixed even though the plants were not subject to short photoperiod after head initiation. The treated plants produced normal spikelets that bloomed normally and produced a normal set of seeds.

Tillering was greatly reduced by short-photoperiod treatment. As short-photoperiod treatment was discontinued after floral initiation, many plants of tillering varieties tillered even at that late stage. Such tillers were not affected by the fact that the main plant had been florally inducted but grew into large tillers similar to those that develop normally.

Another observation of interest is that in an occasional plant, induction of flowering was delayed a day or two as compared with other plants of the same variety. If short-photoperiod treatment was then discontinued, a plant not florally inducted would continue to lay down leaves and would develop into a plant that, to all appearance, had never been subject to short photoperiods.

SUMMARY

Nineteen sorghum varieties and 21 first generation hybrids were grown from midsummer plantings at Chillicothe, Tex., and subjected to both normal and 10-hour photoperiods.

Sorghum is a short-day species, but the varieties in this study exhibited differences in sensitivity to 10-hour photoperiods. Most varieties, including the milos, kalos, hegaris, feteritas, California White durra, Bonar durra, Bonita, shallu, and Freed were sensitive. Manko was a little less sensitive than these varieties but was more sensitive than Blackhull kafir, Sumac, Bishop, and Lemon Yellow. Dwarf broomcorn in both years and Texas Blackhull kafir in the second year were unresponsive to the short-day treatment.

All first generation hybrids were hastened in maturity when subjected to 10-hour photoperiods if one parent was sensitive. Sensitivity to short photoperiod, therefore, is a dominant characteristic. Hybrids that were relatively insensitive to short photoperiods always had two relatively nonsensitive parents.

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IRREGULARITIES IN A HYBRID BETWEEN TRITICUM DURUM AND T. PERSICUM [1]

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INTRODUCTION

It is rather generally believed that, with the exception of crosses involving *Triticum timopheevi* Zhuk., hybrids between species of wheat with the same number of chromosomes are about as normal as individuals of the parent species. However, several writers, including Aase, [2] Thompson and Robertson, [3] and Hosono, [4] have reported irregularities in certain hybrids. During 1941 and 1942 the writer observed various abnormalities in 67 plants of a hybrid between two species of tetraploid wheat at Columbia, Mo. These irregularities are briefly described herein.

MATERIAL AND METHODS

The species of wheat used in the cross were *Triticum durum* Desf. ($n=14$) variety Kubanka and *T. persicum* Vav. ($n=14$). Most of the observations were made on plants of the parents and the hybrid started simultaneously in a greenhouse in early December 1941 and in early February 1942.

Cytological observations were made on acetocarmine smears made permanent by a tertiary butyl alcohol method described by Sears. [5] Photomicrographs were taken of these permanent preparations and of fresh pollen stained with an aqueous solution of iodine.

MACROSCOPIC AND MICROSCOPIC OBSERVATIONS ON PARENTS AND HYBRID

The parents and F_1 plants developed at about the same rate. At maturity the height, number of culms, and seed production of each

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plant were recorded. These data, summarized in table 1, give little indication of heterosis in the F_1 plants as measured by the three criteria. The seed production of the F_1 plants was even less than that of the parents. The reduction probably was due to the greater sterility of the hybrid plants.

The relative fertility of the 2 species and the F_1 hybrid is shown in table 2. The hybrid plants had 3 times the percentage of sterility of *Triticum persicum* and more than 10 times that of *T. durum*.

TABLE 1.—*Average height, number of culms, and seed production of plants of Triticum durum, T. persicum, and the F_1 hybrid*

Stock	Plants observed	Height	Culms	Seed produced
	Number	*Centimeters*	*Number*	*Grams*
T. durum	13	127	4.7	6.66
T. persicum	12	107	6.9	6.96
T. durum × *T. persicum*	12	126	5.7	5.53

TABLE 2.—*Fertility of plants of Triticum durum, T. persicum, and the F_1 hybrid*

Stock	Plants observed	Florets			
		With seeds		Without seeds	
	Number	*Number*	*Percent*	*Number*	*Percent*
T. durum	6	610	98.1	12	1.9
T. persicum	8	840	92.8	65	7.2
T. durum × *T. persicum*	6	388	78.5	106	21.5

Observations on mature pollen revealed a similar relation. About 39 percent of the pollen in the hybrid plants was visibly defective as compared with 11 percent in *Triticum persicum* and 5 percent in *T. durum* (fig. 1 and table 3).

TABLE 3.—*Pollen counts on plants of Triticum durum, T. persicum, and the F_1 hybrid*

Stock	Plants observed	Pollen grains filled with starch as indicated—					
		Well filled		Partially filled		Empty	
	Number	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
T. durum	3	1,185	94.6	21	1.7	46	3.7
T. persicum	2	829	89.1	14	1.5	87	9.4
T. durum × *T. persicum*	3	997	60.6	149	9.1	499	30.3

Cytological observations on microsporogenesis revealed irregularities in the F_1 plants (table 4). The most conspicuous abnormality was a quadrivalent which was present in each pollen mother cell (fig. 2). In about one-third of the pollen mother cells the quadrivalent occurred in the form of a chain. From the observations of Thompson and Thompson [6] it appears that the greater sterility in the hybrid (table 2)

[6] THOMPSON, W. P., and THOMPSON, M. G. RECIPROCAL CHROMOSOME TRANSLOCATIONS WITHOUT SEMI-STERILITY. Cytologia (Fujii Jubilaei Vol.) 1937: 336–342, illus. 1937.

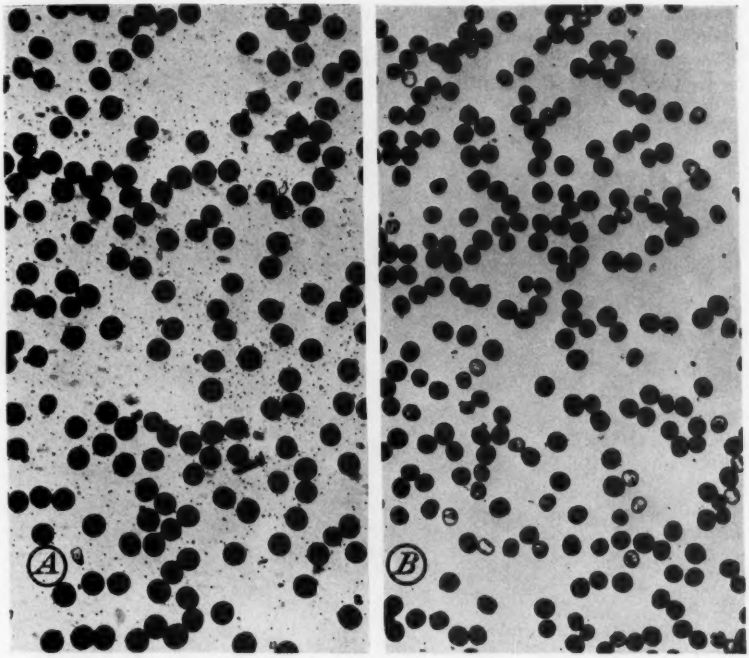

FIGURE 1.—Mature pollen stained with iodine: *A*, *Triticum durum*; *B*, F_1 hybrid (*T. durum* × *T. persicum*). Note that the pollen grains of the hybrid were smaller and that a greater proportion of them were incompletely filled or empty. × 70.

TABLE 4.—*Cytological observations on meiosis in Triticum durum, T. persicum, and the F_1 hybrid*

Stock	Microsporocytes with arrangement of chromosomes as indicated—									Quartets with micronuclei				
	Open bivalents					Univalents		Quadrivalents						
	0	1	2	3	4	0	2	0	1	0	1	2	3	4
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
T. durum	39	18	9	0	0	66	0	66	0	205	11	0	0	0
T. persicum	13	22	7	3	1	46	0	46	0	335	49	14	1	0
T. durum × *T. persicum*	20	19	20	9	1	67	2	0	69	551	45	25	7	

probably cannot be attributed to the quadrivalent. Open bivalents also were more common in the hybrid than in the parents (1.3 per cell as compared with 1.1 per cell in *Triticum persicum* and 0.55 in *T. durum*). Univalents were rare in the hybrid, but none was observed in the parent species. There was no indication of inversions in the

chromosomes of the F_1 plants. Micronuclei in the quartets resulting from the second meiotic division were about equally frequent in *T. persicum* and the F_1 (table 4). In both they were about four times as frequent as in *T. durum*.

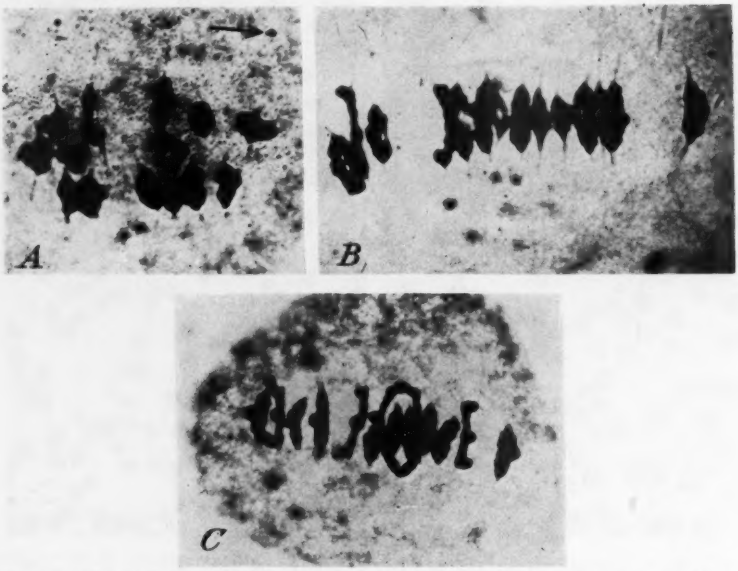

FIGURE 2.—*A*, Pollen mother cell of *Triticum durum* at first meiotic metaphase. Note the 14 closed bivalents and the tiny fragment (arrow) which was also regularly present in this plant. *B*, Pollen mother cell of *T. durum* × *T. persicum* at first meiotic metaphase. Note the chain quadrivalent and the 12 bivalents, of which 1 is open. *C*, Pollen mother cell from the same individual as *B*, showing a ring quadrivalent and 3 open bivalents. × 850.

A fragment similar to that in figure 2, *A*, has been observed by the writer [7] in common wheat.

In addition to the irregularities mentioned, there were other but less definite abnormalities in the F_1 plants. Metaphase plates were not neat and orderly. The chromosomes were not well-defined and regular in outline, and the bivalents and the anaphase chromosomes were frequently stuck together. In general the pollen mother cells of the F_1 plants presented a more disorderly appearance than those of the parent plants, although lagging and loss of chromosomes or formation of bridges at first anaphase did not appear to be any more frequent in the hybrid plants than in the parents.

DISCUSSION

It is evident that the stocks of *Triticum durum* and *T. persicum* used in this study had differentiated in other ways than morphologically. It would be surprising if this were not so. In most cases the accumulation of differences that characterize these and other species

[7] Unpublished observation.

could take place only if aided by geographic or genetic isolation. This differentiation exhibited itself in chromosomal irregularities, partial sterility, and possibly other irregularities in the hybrid plants. Such disorders probably act with linkages in interfering with the transfer of characteristics such as quality, disease resistance, winter hardiness, and yield from one species to another, even though the species have the same number of chromosomes.

In a number of respects *Triticum persicum* was intermediate between *T. durum* and their hybrid. *T. persicum* had more defective pollen, greater sterility, and a higher frequency of meiotic irregularities than *T. durum*. The irregularities in *T. persicum* and the hybrid were probably due to both genetic and physiologic causes.

The apparent absence of heterosis in this species cross is of interest.

SUMMARY

Observations on plants of a hybrid between two tetraploid species of wheat (*Triticum durum* and *T. persicum*) revealed a number of irregularities, although the number of chromosomes in the two species was the same. Meiotic abnormalities included a quadrivalent and other but less readily analyzable peculiarities. Plants of the hybrid exhibited no evidence of heterosis and were partly sterile, possibly because of physiologic as well as genetic factors. It is probable that such irregularities in hybrids between species with the same number of chromosomes are more common than is generally recognized.

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